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Microwave Assisted Synthesis of Phenazines from β-Lapachones and Their Tuberculostatic Activity

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DISO, LFA and RSFS performed the all synthetic work-up of the phenazines, wrote the protocol and wrote the first draft of the manuscript. Author MCSL managed the biological assays and wrote the protocol. Authors VWHR and NCR performed the molecular modeling calculations and wrote the protocol. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study aimed to investigate de synthesis of phenazines by microwave assisted reactions and evaluate the tuberculostatic activity.
Study Design: The method developed in the study is an alternative for the method described in the literature employing acetic acid, an efficient solvent for the reaction, but corrosive and aggressive health. In addition tuberculostatic activities of phenazines were assessed and a brief study on molecular modeling of the phenazines was performed.

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Place and Duration of Study: The experiments were performed on Instituto Federal do Rio de Janeiro (IFRJ), Fundação Oswaldo Cruz (FIOCRUZ) and Núcleo de Pesquisas em Ecologia e Desenvolvimento Social de Macaé (NUPEM) between 2012-2015.
Methodology: Quinone (1 mmol) and 5 mmols of *o*-phenylenediamine were dissolved in 10 ml of 1,4-dioxane in a sealed tube. Microwave reactor conditions were: temperature 150°C and 4.5 bar pressure for 15 minutes. After the reaction water was added to the reaction media and the yellow precipitate formed was filtered under vacuum and washed 3 times with water. The phenazine was purified by silica gel column chromatography using a mixture of hexane-ethyl acetate 95 : 5 as eluent, the phenazine were obtained in yields greater than 85% in all cases.
Results: Six phenazines derived from β-lapachonas were synthesized by microwave assisted reactions with good yields. The tuberculostatic activity of the five phenazines was significantly lower than phenazine from β-lapachone, a brief molecular-modeling study performed showed that the higher activity of phenazine from β-lapachone is due its polarity (μ) and Polar Surface Area (PSA).
Conclusion: The microwave irradiation was effective to synthesize phenazines from β-lapachones faster and with similar yields.

Keywords: β-lapachones; microwave; phenazines; tuberculosis.

1. INTRODUCTION

Phenazines are nitrogen-containing heterocycles which basic structural moiety is the 5-10-diaza-anthracene. Phenazines can be obtained from natural source or organic synthesis [1]. Natural phenazines are isolated from bacteria, mainly genera *Pseudomonas* [2] and *Streptomyces* [3-5].

There are several methods to phenazine synthesis, some methods were developed in the early twentieth century such as Wohl-Aue synthesis [6] and Bemberger-Ham synthesis [7], other method developed in the 70's was the Beirut reaction [8]. These methods provide the phenazine moiety in the mono or di *N*-oxide form which must be reduced to furnish the phenazine. Phenazines can be synthesized directly, without requiring reduction reaction, by cross-coupling reactions palladium catalyzed [9] or by condensation reaction between *o*-phenylenediamines and *o*-naphthoquinones, (Fig. 1).

The condensation reaction is very efficient and was developed by Samuel Hooker in the nineteenth century [10] for synthesis of several phenazine derivatives from β-lapachone and analogues. These phenazines have shown antimalarial and tuberculostatic activities [11-14] this method employs a reflux reaction in acetic acid and it presents almost quantitative yields (higher than 95%) however the acetic acid is corrosive and can cause skin burns, permanent eye damage, and irritation to the mucous membranes. Condensation reactions using acetic acid in dichloromethane [15] and in the neutrals media [16] were also effective.

Microwave assisted reactions represented an advanced in organic synthesis in the last 30 years, the microwave irradiation can provide higher yields and faster reaction times which may result from at least three effects:

Microwave thermal effects of are those obtained by high temperatures that can be obtained by microwave irradiation, the speed up on chemical reactions at high temperatures follows the behavior predicted by the Arrhenius law ($k = A.e^{-Ea} / RT$). It is important to note that in this case the reaction has the same result as that when it is performed under conventional heating, only this result is obtained in considerably shorter times.

Microwave specific thermal effects are those that cannot be reproduced under conventional heating, it may be related the facts that it is possible overheating of solvents higher than their boiling point and microwave irradiation promotes higher heating rate which allows reach high temperatures faster than conventional heating. Especially in modern reactors, the pressure also takes important part in these effects since it can perform overheating both atmospheric pressure and higher pressures.

Microwave non-thermal effects are those that can not be explained by increase of temperature whereas today microwave reactors allows to perform microwave assisted reactions even under negative temperatures. These effects are associated with decreased activation energy by the transition state stabilization or an increased

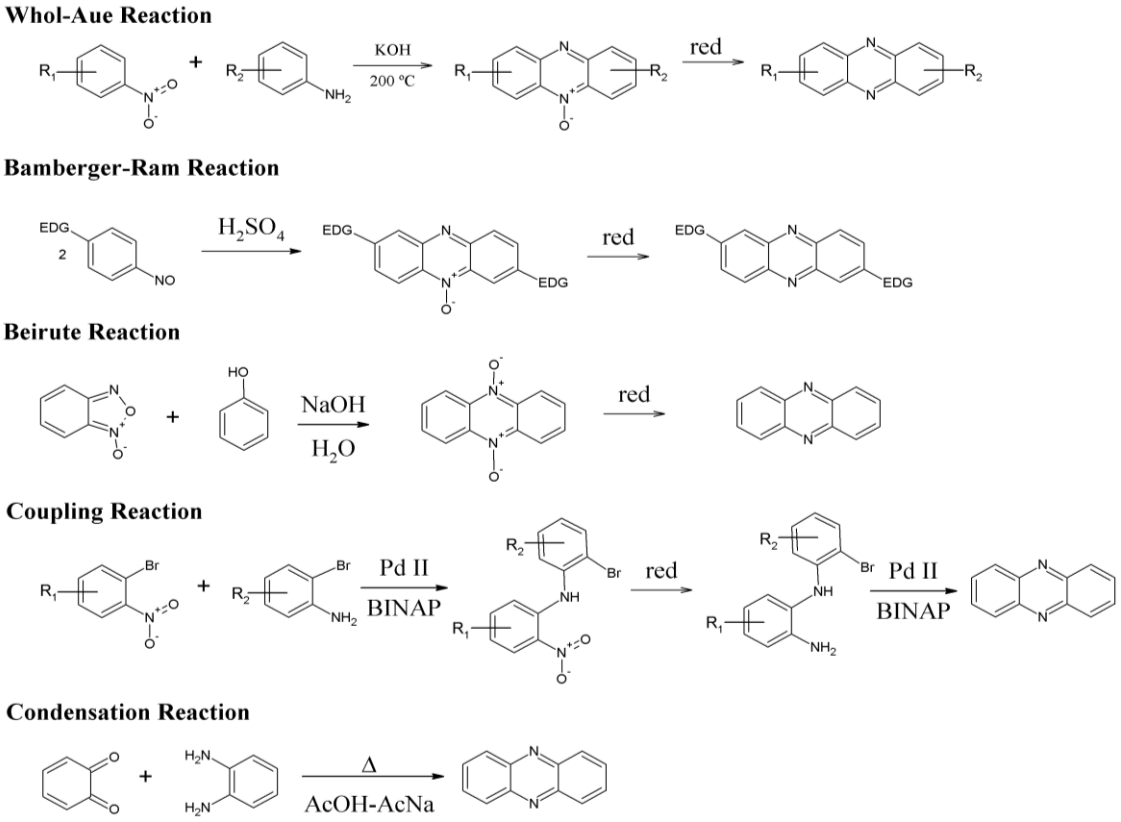


Fig. 1. Synthetics methods of phenazines

probability of molecular shocks, the two effects are the result of electric fields generated by microwave irradiation.

Theoretical aspects of microwave irradiation effects on chemical reactions are didactically approached and explained in two reviews [17-18].

Considering the tuberculostatic activity presented by phenazine from β-lapachone [19] also named lapazine, the aim of this study was to develop a microwave assisted method to synthesize lapazine and five other phenazines from β-lapachone analogues already described in the literature [20], which they were prepared by condensation reaction under conventional heating. Although six compounds are not sufficient to perform a significant structure-activity relationships (SAR) study, some molecular parameters were calculated by molecular modeling for the phenazines.

2. MATERIALS AND METHODS

Quinones **1-6** used as starting materials and chromatographic standards of phenazines **7-2**, which were synthesized in this work, were gently provided by the Chemist Maria do Carmo F. R. Pinto from Laboratório de Química Heterocíclica Antonio Ventura Pinto on Instituto de Pesquisa de Produtos Naturais of Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil.

However, methods to synthesize quinones **1-6** and physical data are available in the literature [20-24]. Preparation of phenazines **7-12** and physical data are available in the literature [11,20]. The products of the reactions were identified by TLC with standard samples of phenazine and melting point comparison with the literature.

2.1 Microwave Assisted Synthesis of Phenazines

The microwave assisted reactions were performed in a microwave reactor model MONOWAVE 300 (Anton Paar, Austria). General experimental procedure to phenazine synthesis is described below:

Quinone (1 mmol) and 5 mmols of o-phenylenediamine were dissolved in 10 ml of

1,4-dioxane in a sealed tube. Microwave reactor conditions were: temperature 150°C, 10 W and 4.5 bar pressure for 15 minutes. After the reaction water was added to the reaction media and the yellow precipitate formed was filtered under vacuum and washed 3 times with water. The phenazine was purified by silica gel column chromatography using a mixture of hexane-ethyl acetate 95 : 5 as eluent, the phenazine were obtained in yields greater than 85% in all cases.

2.2 Evaluation of Tuberculostatic Activity and Minimum Inhibitory Concentration (MIC)

The tuberculostatic activity of Lapazine was already published on literature [19], the tuberculostatic activity of phenazines **8-12** were assayed in this study follow the protocol described below:

To test its antimicobacterial activity, the primary screening was conducted at 100 $mg.mL^{-1}$ against *M. tuberculosis* (ATCC 27294 $H_{37}R_v$) in BACTEC12B medium using the Microplate Alamar Blue Assay (MABA) [25]. Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system [26]. Compounds showing 90% inhibition in the primary screening were considered active, and then retested at a lower concentration against *M. tuberculosis* (ATCC 27294 $H_{37}R_v$) in order to determine the actual MIC, using MABA. Rifampicin was used as the reference compound (MIC 1.0 $mg.mL^{-1}$).

2.3 Molecular Modeling Methods

First, the molecules were built with Spartan‘10 v. 1.1.0 (Spartan'10 Tutorial and User's Guide. Irvine, California: Wavefunction, Inc.) and then submitted to conformational analysis with MMFF Force Field [27], followed by geometry optimization with RM1 semi empirical [28]. Finally, a single point calculation was performed with HF 6-311G* Basis Set for the calculation of stereo electronic properties. Descriptors' calculations were performed either in Spartan‘ 10 v. 1.1.0 or in Osiris Property Explorer (http://www.organicchemistry.Org/prog/peo).

3. RESULTS AND DISCUSSION

This study aimed mainly the synthesis of phenazines from β-lapachone by microwave irradiation in acid acetic free conditions. Although the condensation reactions in glacial acetic acid works very well for those synthesis, the acetic acid is irritant to mucous, eyes e skin. It is also corrosive for metals and flammable liquid and vapour. Initially, were tested solvents ethanol, methanol, THF and 1,4-dioxane. The 1,4-dioxane promoted the complete precipitation of the phenazine after addition of a aqueous saturated solution of NaCl, so 1,4-dioxane was chosen. The others solvents promoted a partial precipitation, which demanded evaporation of ethanol, methanol and THF, dissolution of residue in ethyl acetate followed by water washing and evaporation of ethyl acetate for isolation of phenazines, so the 1,4-dioxane was selected as solvent for the reactions. However, very close final yields were obtained with all solvents after purification by column chromatography.

The conditions to microwave assisted synthesis of phenazines 7-12 from quinones 1-6 are summarized on (Fig. 2).

The yields of reactions were good and similar to the yields obtained by condensation reaction in acetic acid under reflux. The reaction time was eight times faster than condensation reaction.

The solvent employed in the reaction, 1,4-dioxane, has boiling point 100ºC, the reactions undergone at temperature 150ºC, fifty percent higher than its boiling point, the heating rate were more than 50ºC. min^{-1}, the pressure in the reaction media was 4,5 bar, The reactor MONOWAVE 300 was used in the constant temperature mode, in this mode the equipment radiates microwave pulses to maintain the selected temperature.

These parameters are impossible to be obtained by conventional heating, so the catalytic effect observed in this reaction can be attributed to specific microwave thermal effects.

The tuberculostatic activity of phenazine **7** which is synthesized from β-lapachone was recently published [19], it showed MIC of 3,0 $\mu g.mL^{-1}$ in addition it was active against a Rifampicine resistant strain of *Mycobacterium tuberculosis*. These result lead us to perform assays to evaluate the tuberculostatic activity of phenazines **8-12** synthesized by microwave irradiation, the results are shown in Table 1.

As shown in Table 1, phenazine **7** is almost ten times more active than the brominated

phenazine **8** and the others phenazines are practically inactive. The significant difference on tuberculostatic activity for phenazine **7** in relation to other, led to perform a brief molecular modeling study for physical-chemical parameters for phenazines. Table 2 shows selected calculated descriptors for the molecules in this study.

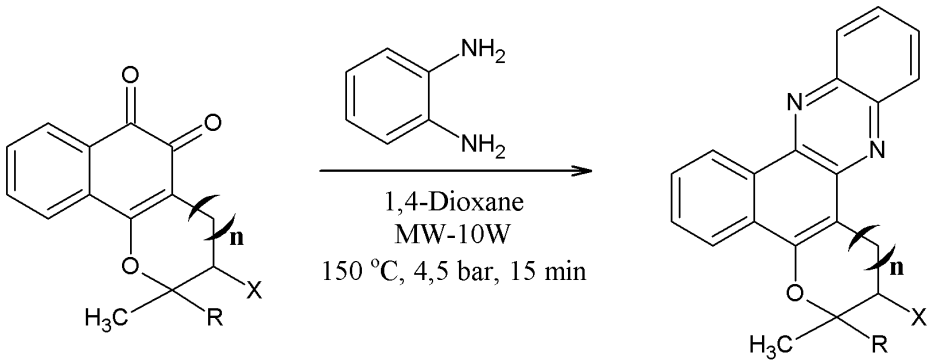
Overall, phenazine **7** showed a smaller Polar Surface Area (PSA) compared to the inactive ones, with the exception of **8**, which has a higher PSA value which could be related to the activity reduction. Also, there is a small correlation between lipophilicity, water solubility and the biological activity, suggesting that higher lipophilicity and lower water solubility enhance the activity of the compounds under study.

Table 1. Activity of phenazines against *Mycobacterium tuberculosis*

Phenazine	MIC (μg.mL^{-1})
7	3.0*
8	25
9	>100
10	>100
11	50
12	>100
Rifampicine	1.0

**Ref. [19]*

In general, introduction of electronegative groups, -Br, -OH or -SO_3H led to a loss of activity, moreover, although phenazines **11** and **12** have similar PSA to phenazine **7**, substitution of the pyran ring by furan led lower polarity and consequently to loss of activity, suggesting that



Quinone	n	x	R	Phenazine	Yield (%)
1	1	H	CH_3	**7**	90
2	1	Br	CH_3	**8**	90
3	1	OH	CH_3	**9**	85
4	1	SO_3H	CH_3	**10**	86
5	0	H	CH_3	**11**	89
6	0	H	H	**12**	87

Fig. 2. Microwave assisted synthesis of phenazines from β-lapachones

Table 2. Physical-chemical parameters calculated for phenazines 7-12*

Phenazine	MW (Da)	HOMO (eV)	LUMO (eV)	μ (Debye)	Volume (Å^3)	PSA (Å^2)	cLogP	LogS
7	314.39	-7.68	1.00	1.21	327.13	18.48	4.92	-5.55
8	393.28	-7.91	0.81	1.91	345.42	18.61	5.34	-6.02
9	330.39	-7.75	0.94	0.91	337.53	37.24	4.07	-5.15
10	394.45	-7.99	0.75	3.50	364.97	71.76	2.68	-4.70
11	300.36	-7.53	0.93	0.58	311.07	20.64	4.58	-5.28
12	286.33	-7.54	0.91	0.51	293.08	21.08	4.28	-5.17

** MW= Molecular Weight; HOMO= Highest Occupied Molecular Orbital; LUMO= Lowest Unoccupied Molecular Orbital; μ= Dipole Moment; PSA= Polar Surface Area; cLogP= Logarithm of the Partition Coefficient; LogS= Logarithm of the Solubility*

this ring is not favorable for the biological activity. Furthermore, HOMO and LUMO distribution and density maps and electrostatic potential maps were calculated to gain more insight on other molecular properties contributions to the biological activity.

HOMO and LUMO distributions are very similar for all the molecules, which indicate that they are not significant for the biological activity. It is worth mentioning that, in the analysis of the LUMO density map, it can be observed that two carbon atoms in the C ring have electron-poor characters and therefore can be liable to suffer nucleophilic attack. However, as the same feature can be observed in all molecules, one may hypothesize that these sites may be auxiliary but not activity-enhancing. In the HOMO density maps, we can observe a HOMO density distribution located on the C ring in all molecules in the series.

As illustration, Fig. 3 shows the HOMO-LUMO distribution and density for active phenazine **7**.

In the analysis of the electrostatic potential maps (Fig. 4) it was observed a similar distribution of charges for all molecules. For molecules **8**, **9** and **10**, charge polarization occurs in the substituent region, since there are regions of low and high electron density. However, in molecule **8** there is not a significant polarization as observed for molecules **9** and **10**, which may explain its higher activity.

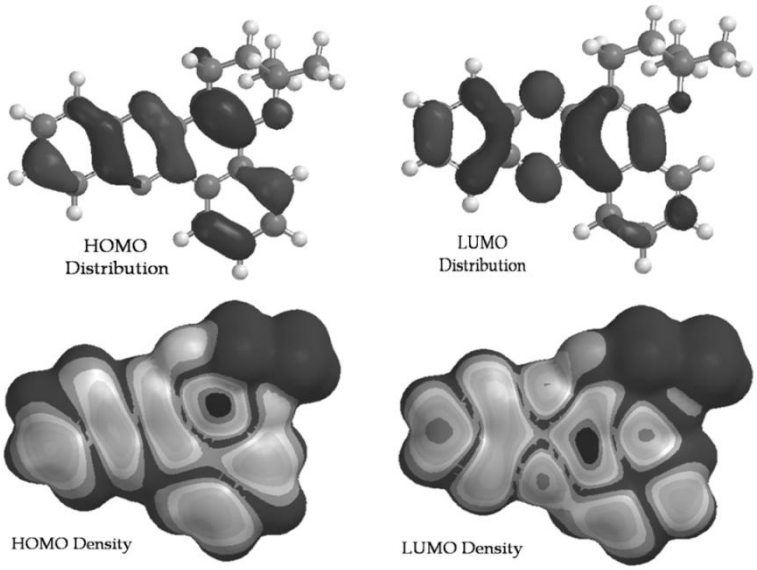


Fig. 3. HOMO-LUMO density for phenazine 7

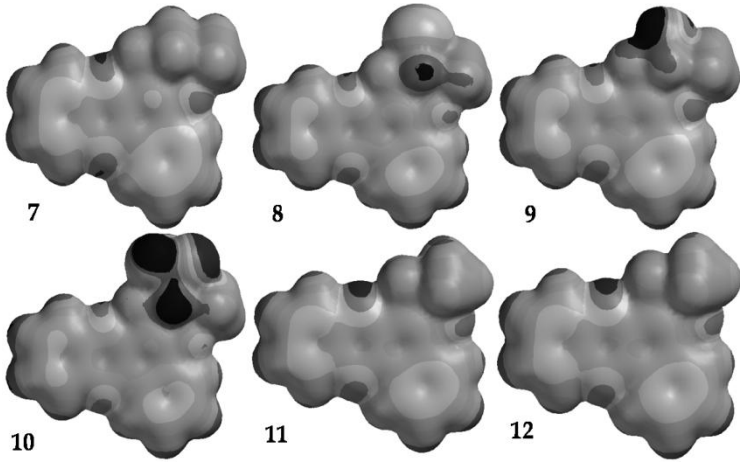


Fig. 4. Electrostatic potencial maps for phenazines 7-12

In phenazine **7**, there is low electron density in the substituent region, which may be associated with interactions with regions of high electron density in the bioreceptor. The inclusion of groups that significantly increase the electron density in this region led to a drastic reduction in the activity of the molecules, like phenazines **9** and **10** for example.

Anyway, although it was desirable a study with a larger number of molecules, the proposal here was a brief study that could explain the much higher activity of phenazine 7 in comparison to the other five ones synthesized in this study.

4. CONCLUSION

The microwave irradiation was effective to synthesize phenazines from β-lapachones faster and with similar yields those achieved with conventional reactions and without toxic acetic acid. The tuberculostatic activity of the five phenazines synthesized in this work were significantly lower than phenazine from β-lapachone, a brief SAR study performed showed that the higher activity of phenazine from β-lapachone is due its polarity (μ) and Polar Surface Area (PSA).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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