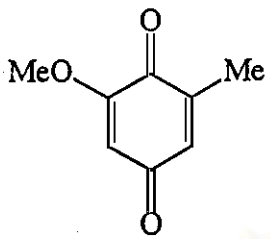
CHAPTER I
INTRODUCTION

The biological study of synthesized compounds with useful properties, and of some derivatives and synthetic analogs, furnished the early hypotheses about possible relations of chemical structures and over-all biological behavior of these substances. Modifications of the basic structures are obtained by chemical synthesis, and the effects of these changes on biological response are used to compile structure-activity relationships (SAR). The SAR study is therefore interesting research since it is a challenge way to find bioactive compounds. Both physical and chemical properties of synthesized compounds may have a bearing on the biological activity. Examples of such properties include solubility, partition coefficients, degree of ionization, surface activity, isosterism, intermolecular forces, interatomic distances between functional groups and stereochemistry. More commonly it is found that compounds that are unrelated chemically may have the same action, and that compounds with the same functional groups possess widely different biological activities. Such divergence from established structure-activity relationships requires the synthesis of a large number of compounds before a useful agent can be found.

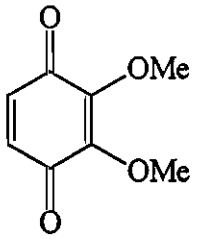
1.1 Introduction to quinones

Quinones could be categorized into three types according to their structures :

1. Benzoquinones For example 6-methoxy-2-methyl-1,4-benzoquinone; $C_8H_8O_3$ (a) has been found in cultures of an unidentified *Aspergillus sp.*[1] It has been synthesized by oxidation of 6-methoxy-2-methylphenol with peracetic acid and of 2-amino-3-methoxytoluene with Fremy's salt.[2]



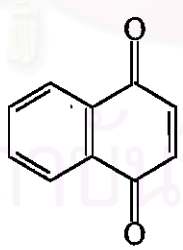
(a)



(b)

2,3-Dimethoxy-1,4-benzoquinone; $C_8H_8O_4$ (**b**) has been found in the defensive secretion of the millipedes *Uroblaniulus canadensis*, *Metiche tanganyicense*, *Pachybolus brachysternus*, *Ophistreptus levis*, and *Spirostreptus pavani*.[3] This compound is synthesized from 2,3-dimethoxyphenol by oxidation with Fremy's salt[4] or coupling with diazotized sulphanilic acid, reduction with dithionite, and oxidation of the aminophenol with chromic acid.[5]

2. Naphthoquinones such as 1,4-naphthoquinone; $C_{10}H_6O_2$ (**c**) was identified in the defensive secretion of the opilionid *Phalangium opilio* by GC-MS and has been observed in several *Juglans* spp. where it is an established intermediate in the biosynthesis of juglone in *Juglans* regia. A monoglucoside of the hydroquinone is also present.[6]



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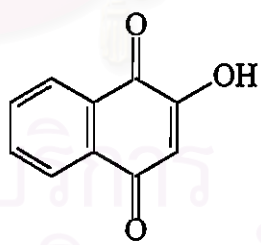
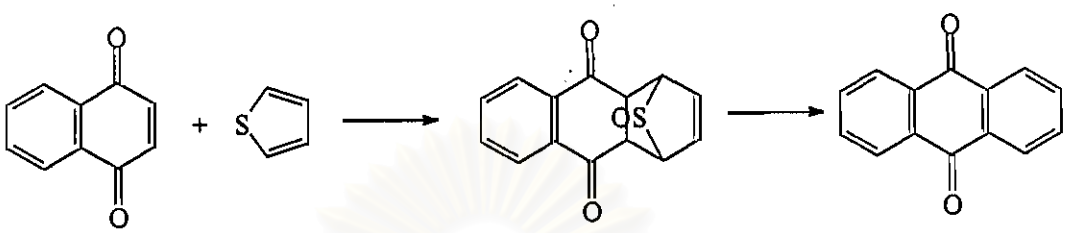


(d)

Lawsone; $C_{10}H_6O_3$ (**d**) was found as the sodium and calcium salts in seeds of *Lomatia ferruginea*[7] and it was the colouring principle of henna, probably the oldest cosmetic known to man' which has recently returned to fashion as a hair dye.

3. Anthraquinones such as 9,10-anthraquinone; $C_{14}H_8O_2$ (**e**) has been reported several times from natural sources, probably as an artefact, but it is a genuine natural product in the leaf wax of perennial rye grass (*Lolium perenne*).[8] Commercial

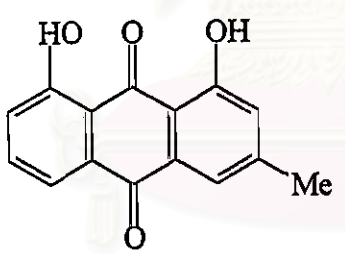
syntheses of this important compound are well known. A chemically interesting procedure is shown below.[9]



(e)

Treating a mixture of 1,4-naphthoquinone and thiophene in methylene chloride with *m*-chlorobenzoic acid at 0 °C for two days gave (e) in 25% yield. The intermediate adduct was not isolated. 2-Methylanthraquinone can be obtained similarly in lower yield from 3-methylthiophene.

Other two well-known anthraquinones are chrysophanol; $C_{15}H_{10}O_4$ (f) and rhein; $C_{15}H_8O_6$ (g) those were found in roots of *Abrus cantoniensis* and in flowers of *Cassia marginata* (both Leguminosae), respectively.[10]



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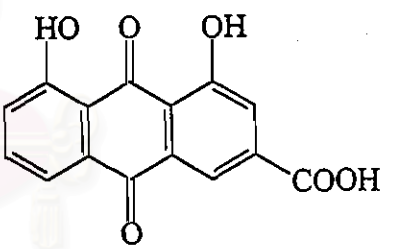
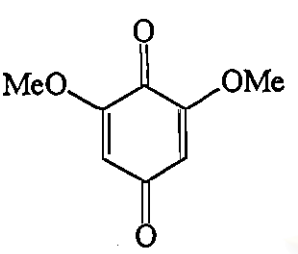


(g)

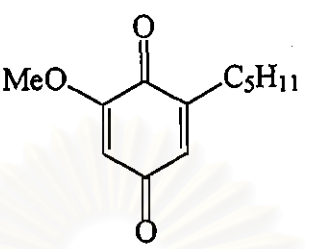
1.2 Literature survey on *p*-benzoquinones and hydroquinones

Naturally occurring *p*-benzoquinones were found in many sources and had been reported to possess many activities. For instance 2,6-dimethoxybenzoquinone (h) was found in the smooth green bark of *Populus balsamifera*, the brown bark of *P. trichocarpa*[11], *Eurycoma longifolia*.[12] Compound (h) was very active against streptococci, staphylococci, and *E. coli* but it had no effect on *Ps. Aeruginosa* or *Proteus vulgarus*.[13] The quinone primin (i), then described as 2-methoxy-6-*n*-pentyl-1,4-benzoquinone, was first identified in primrose leaves (*Primula obconica*) and later in the bark of miconia root (*Melastomaceae*). Since the oxidation of the natural extract of the miconia root with aqueous ferric chloride generated primin, the reduced form

was correspondingly called miconidin (j) with the label 2-methoxy-6-*n*-pentyl-1,4-hydroquinone. Both (i) and (j) can be used as antimicrobial agents.[14]



(h)



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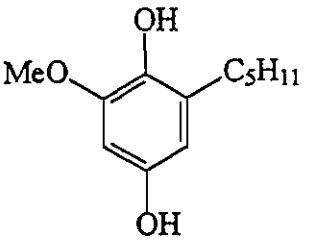
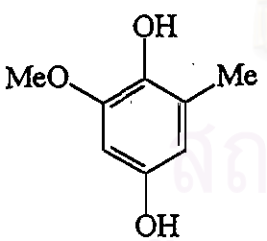
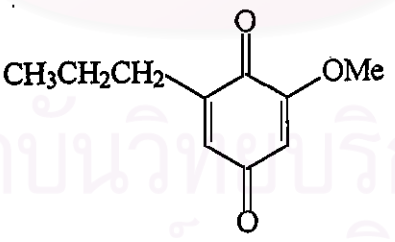


(j)

6-Methoxy-2-methylhydroquinone (**k**) and 6-methoxy-2-methylbenzoquinone (**a**) were intermediate in the penicillic acid biosynthesis in *Penicillium cyclopium*, which has been confirmed by using ^{14}C-labelled precursors demonstrating in the following sequence of the biological formation of penicillic acid in *Penicillium cyclopium*: orsellinic acid ⟶ 2-O-methylorsellinic acid ⟶ (k) ⟶ (a) ⟶ penicillic acid.[2] Four antibiotic compounds were 2-methoxy-6-(1-propyl)-1,4-benzoquinone (**l**), 2,3-dimethoxy-5-(1-propyl)-1,4-benzoquinone (**m**), and their corresponding hydroquinones which have been isolated from the culture medium of the fungus *Camarops microspora* (Karst.) Shear.[15]



(k)



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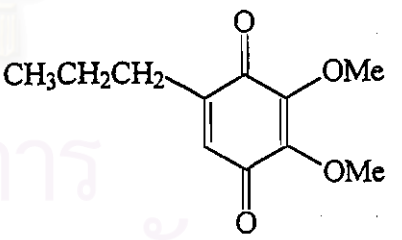
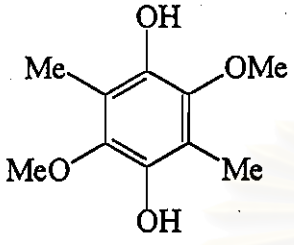


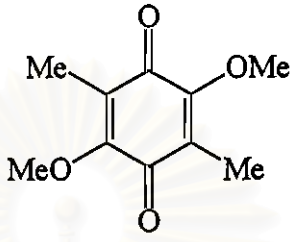
(m)

From the crude extracts of *Nectria coryli*, it was found 2,5-dimethoxy-3,6-dimethylhydroquinone (**n**) and the corresponding quinone (2,5-dimethoxy-3,6-dimethylbenzoquinone, (**o**)). Both (**n**) and (**o**) inhibited the growth of *Staphylococcus aureus*, at a concentration of 1 μg per mL and the natural occurrance (**n**) and (**o**) in the same organism is of interest in connection with the demonstrated coenzyme Q activity of (**n**).[16] The occurrance of natural products having the 3-alkyl or 3-allyl substituted 2,5-dihydroxy-1,4-benzoquinone structure and their applicaton, *e.g.*, as anthelmintic

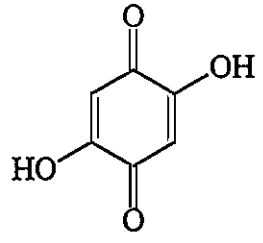
agents, was taken to elaborate a general procedure for the introduction of alkyl groups into 2,5-dihydroxy-1,4-benzoquinone (**p**). The reaction of organoboranes with (**p**) gave 2,5-dihydroxy-1,4-benzoquinone derivatives.[17]



(**n**)

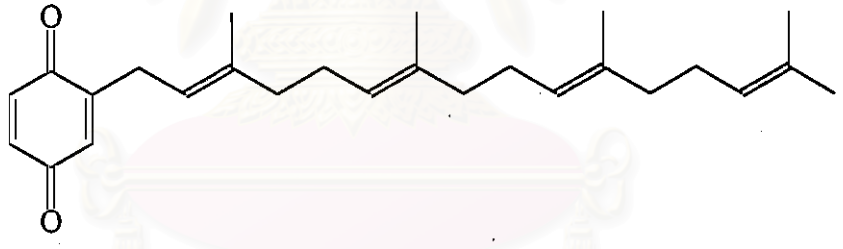


(**o**)

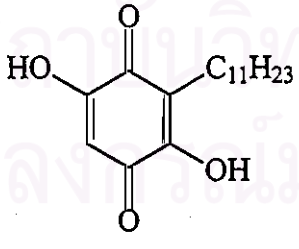


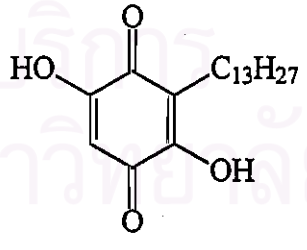
(**p**)

2-Tetraprenylbenzoquinone (**q**) was isolated from *Ircinia muscarum* which is a kind of marine sponges.[18] Extractives of the bark of *Aegiceras corniculatum* (Stickm.) Blanco provided embelin (**r**) and rapanone (**s**).[19] The two substances (**r**) and (**s**) revealed antiinflammatory, but (**s**) was more potent than (**r**).



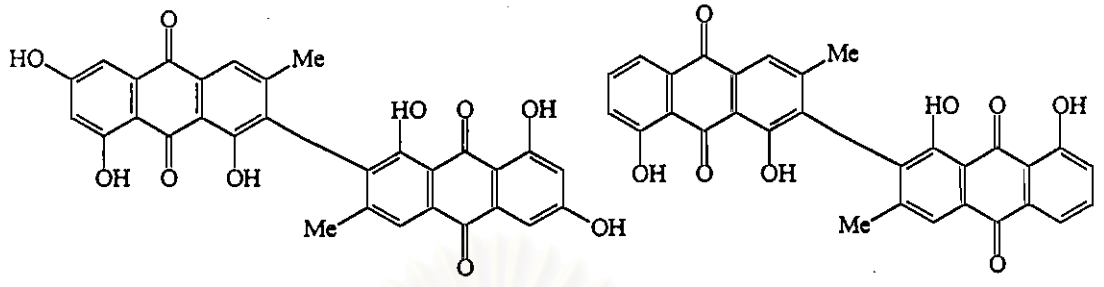
(**q**)





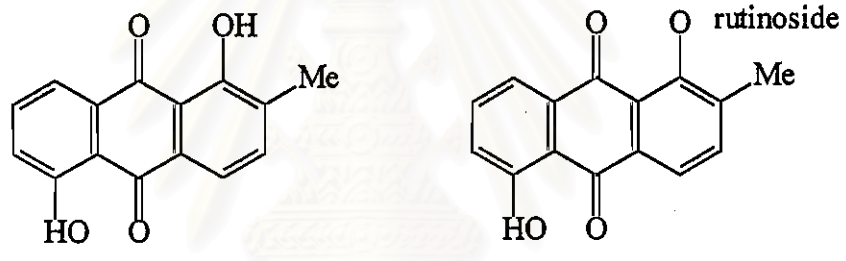
(**r**) (**s**)

Other types of quinones abound in nature having interesting bioactivities such as cassiamin B (**t**) and C (**u**) isolated from the root bark and trunk bark of *Cassia siamea*[20] are shown to be the symmetry dimers of emodin and chrysophanol, respectively, linked in the 2,2′-positions.



(t) (u)

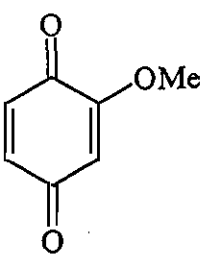
The rare anthraquinones 1,5-dihydroxy-2-methylanthraquinone (v) and 5-hydroxy-2-methylanthraquinone-1-O-rutinoside (w) were isolated from the stems of *Cassia alata*.[21]



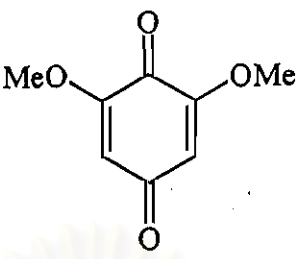
(v) (w)

1.3 Structure activity relationship (SAR) study of benzoquinones

Structure activity relationship (SAR) study of natural products has recently become more attractive to scientists. The main benefit is to gain comprehensive understanding what part(s) of the molecule affect the activity. Furthermore, the design of a model of potential bioactive compounds should stem from this point. There were some reports related to the SAR study of benzoquinone. For example, in 1963[22], 2-methoxy-1,4-benzoquinone (I), 2,6-dimethoxy-1,4-benzoquinone (II), and 2-methoxyhydroquinone (III) were synthesized and their relative toxicity and mode of toxic action on *Ustilago nuda* and *U. tritici* spores and mycelium were investigated. All compounds were highly toxic to *U. nuda* and *U. tritici* spores. The most toxic compound was I, while III and II are less toxic in that order.



(I)



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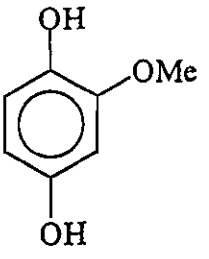
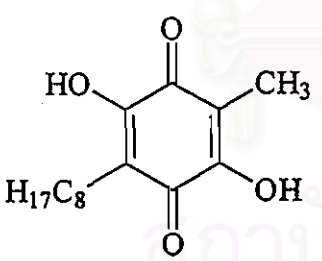


(III)

The toxic action of these three compounds on loose smut spores and mycelium was fungicidal rather than fugistatic. S. Hayashi *et al* [23] studied the relationship between antitumor and antibacterial activities of 16 quinone derivatives. They found no relationship between them (tested against Ehrlich ascites cells, *Staphylococcus aureus* and *Escherichia coli*). Inhibition of respiration of rat liver intact mitochondria of alkyldihydroxy-*p*-benzoquinone and related compounds were also examined.[24] It was found that 2-methyl-5-octyl-3,6-dihydroxy-1,4-benzoquinone (IV) and ardisiaquinone B (V) showed specific inhibition of State-3 respiration but in high concentration. They inhibited both State-3 and State-4 respiration.



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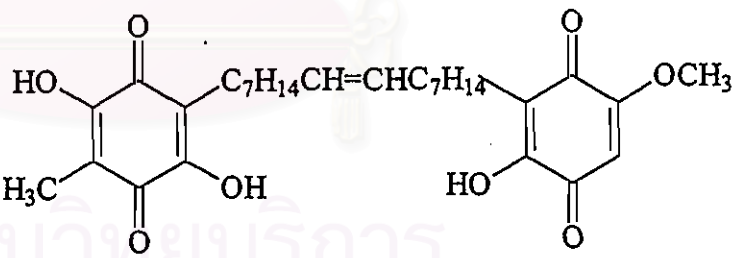
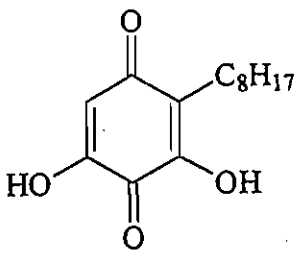


(V)

Both 2-octyl-3,5-dihydroxy-1,4-benzoquinone (VI) and ardisiaquinone A (VII) inhibited State-3 and State-4 respiration.



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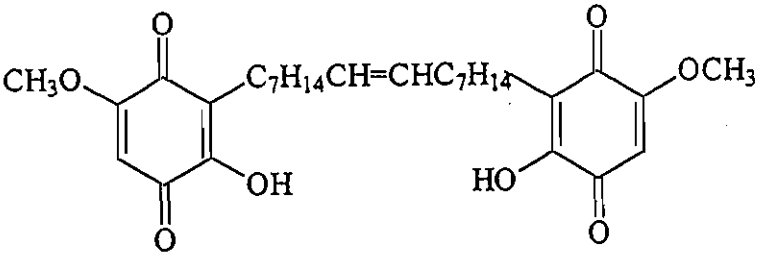
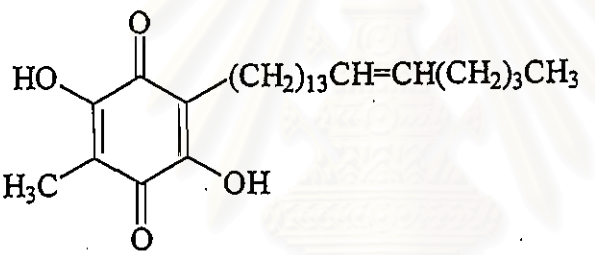
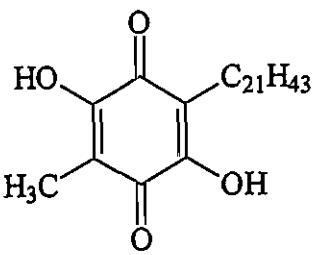


(VII)

However, rapanone (s), maesaquinone (VIII), polygonaquinone (IX) and related compounds having longer alkyl chains did not show any effect. As the result, carbon numbers of the side chain have been found to be an important factor to exhibit the activity.

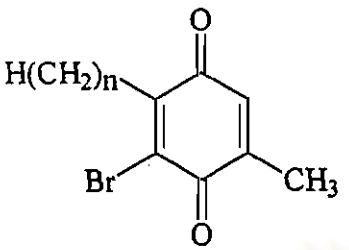


(VIII)



(IX)

From literature search revealed that the SAR study of haloquinones has rarely studied. Only one publication concerning with bromoquinone derivatives was reported and showed the relationship between the structure and inhibitory characteristic in mitochindria.[25] In that paper, four classes of the analogues of bromoquinone, are synthesized. They were 5-bromo-3-methyl- (X), 3-bromo-2-methoxy-5-methyl- (XI), 2,5-dibromo-3-methyl- (XII), and 3,5-dibromo-2-methyl (XIII) derivatives with the side chain $-(CH_2)_nH$; where n = 5, 7, 9, 11 and 13 at position 6. Dibromo compounds usually displayed more inhibitory effect in the mitocondrial ubiquinol cytochrome *c* reductase. The maximal inhibition was found to be 9 methylene groups as a functional group.



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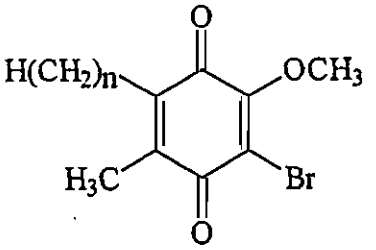

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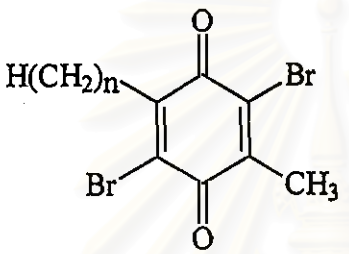

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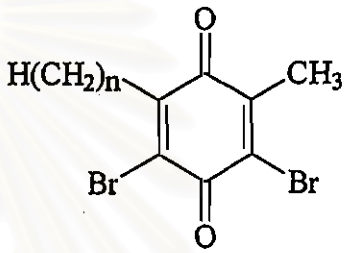

(XIII)

1.4 Bioactivity assay

4.1 Brine Shrimp Lethality Test (BSLT) [26]

Brine shrimp lethality test is a procedure for general toxicity screening. It does not require too much specialization; therefore it is essential as a preliminary testing in the study of bioactive compounds. This procedure is rapid, convenient, reliable, inexpensive, sensitive and it requires little material and is able to identify a broad spectrum of activities. A simple animal that has been used for this technique is the brine shrimp (*Artemia salina* Leach). It has been proposed as a standard test by B. N. Meyer *et al*[26] The aim of this method is to provide a front-line screen that can be backed up by more specific and more expensive bioassays.

Life cycle of *Artemia salina*[27]

Brine shrimp is a crustacean subclass Branchiopoda order Anostraca and family artemidae. Eggs of the brine shrimp are tiny brown particles and have a diameter about 0.2 mm. These are inactive or resting. These eggs have a high resistance to extreme conditions and may be stored for long periods. When they are in seawater (or a saline solution) the eggs adsorb water. It begins to embryo and is completed between 16 to 36 h after immerse in a saline solution. The embryo emerges from the shell still covered in a hatching membrane, and becomes an active, free

swimming nauplius. These larvae are in the instar I or instar II (older) stages. They are 0.4-0.52 μm length, 0.01 mg weight. The colour of the larva is red. There are three pairs of appendages: the antennulae, the antennae and the mandibles. The antennae are used for moving. The larva grow through about 15 stages. The growing larva may be fed yeast cells or unicellular algae. In about 20-35 days, the animal reaches a length of 8.5-9.5 mm and is sexually mature. Thus, in all stages of growth of the brine shrimp, active life is indicated by movement of some appendage or other. The lethal concentration for 50% mortality after 6 h of exposure, the acute LD_{50}, or after 24 h of exposure, the chronic LD_{50}, is determined as the measurement of toxicity of the compounds. LD_{50} or median lethal dose is statistically derived single dose of a substance that can be expected to cause death in 50% of the animals.

1.5 Research objectives

The objectives of this work are to synthesize and to characterize halobenzoquinones and halohydroquinones. All identified compounds were tested for the cytotoxicity to Brine Shrimp Lethality (BSL). The structure and activity relationship of those tested molecules will be concluded.