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Synthesis and SAR of naphthoquinone derivatives as a novel class of algicides against harmful algal species

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유해조류 제어를 위한 naphthoquinone계열 살조물질 합성 및 구조 활성 분석

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이논문을 공학석사 학위신청 논문으로 제출함

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LIST OF ABBREVIATIONS

A.sp.: Amphidinium sp.

CAN: Cerium (IV) ammonium nitrate

$CDCl_3$: Choloroform

DMSO: Dimethylsulfoxide

DMNQ: Dimethoxy-1,4-naphthoquinone

HABs: Harmful algal blooms

Hz: Hertz

LAH: Lithium aluminium hydride

IC_{50}: The half maximal inhibitory concentration

NQ: Naphthoquinone

NFRDI: National fisheries research & development institute

Ppm: Parts per million

THF: Tetrahydrofuran, anhydrous

TMS: Tetramethylsilance

TLC: Thin layer chromatography

초 록

유해조류 제어를 위한 naphthoquinone 계열 살조물질 합성 및 구조 활성 분석

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Quinone 은 자연에 광범위하게 분포되어 있으며 생물학적으로 높은 활성을 보이고 있다. 또한 quinone 은 환경오염원이기도 한 반면 일부 quinone 류는 항암제로 사용되기도 한다. 특히 quinone 은 많은 약품에서 골격구조를 이루며 anthracyclines 와 mixtoxantrones 등은 항암제로 사용되고 있으며 살조제 개발을 위한 연구 또한 진행되고 있다.

Harmful algal blooms (HABs)은 미세조류의 종류 가 수중에서 급속하게 성장할 때 발견 될 수 있으며 이러한 현상은 환경, 식물 및 동물의 건강을 해칠 수 있다. 많은 과학자들은 생리학과 생태학 연구로부터 HABs 가 일으키는 어업의 피해 정도를 감소시키기 위해 많은 노력을 기울이고 있다. 현재 대안으로 사용되고 있는 황토살포법은 환경문제를 다소 줄일 수 있으나 뿌려진 황토가 다시 침강하여 수중 하층부의 산소를 고갈시켜 또 다른 환경문제를 야기할 가능성이 있다. 따라서 생태학적으로 안전하고 선택적인 살조물질을 개발하는 것이 필요하다. 본 연구에서는 40 종의 naphthoquinone (NQ) 유도체를 합성 하였으며,

미세조류에 대한 in-vitro 활성실험을 측정하였다. 대부분의 NQ 유도체들은 유해조류(*Microcystic aeruginos, Anabaena flos-aquae, Stephanodiscus hantzschii, Cyclotella, Peridinium bipes*)에 대하여 활성을 나타냈으며, 무해조류(*Aulacoseira granulate and Scenedesmus actus*)에 대해서는 상대적으로 낮은 독성을 보였다. 특히 화합물 4c-6, 4c-2, 5c-6 및 5c-2 은 5 종의 유해조류에 대해 높은 활성을 보였으며, 1 μM 보다 낮은 IC_{50} 값을 보였다. 이중에서도 화합물 5c-2 는 가장 높은 활성을 보였으며, IC_{50} 값은 0.04-0.78 μM 로 나타났다. 이러한 결과는 NQ 유도체가 유해조류에 대한 살조제로 사용할 수 있는 높은 가능성을 보여주고 있다.

ABSTRACT

Synthesis and SAR of naphthoquinone derivatives as a novel class of algicides against harmful algal species

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Quinones are widely distributed in nature and play essential biological roles. They also occur as substances of potential toxicological significance in environmental pollutants, and some are used as anticancer drugs. Chronic exposure to quinones is mutagenic to cultures of eukaryotic cells and may cause cell death. Considerable interest has been focused on quinone compounds, especially, quinone moieties are present in many drugs a backbone structure, such as anthracyclines and mixtoxantrones used as antitumor agent and algicides.

Harmful algal blooms (HABs) can occur when certain types of microscopic algae grow quickly in water, forming visible patches that may harm the health of the environment, plants, or animals. Green tide algae cause severe problems, such as hindering boat traffic, blocking approaches, obstructing wash processes and catching cavities, creating unattractive foul-smelling loads and killing natural biota. Many scientists have conducted physiological and ecological studies in the hope of reducing the extent of damage to fisheries caused by HABs. The application of clay can treat the environmental problems but the toxins released from flocculated cells and the adverse effect on other organisms.

Therefore, design of ecological safe and selective algicides has been an evolving research topic. In this research, we synthesized 40 naphthoquinone (NQ) derivatives (Table 1) and invitro inhibitory activity was measured against microalgae. All the synthesized compounds were showed invitro inhibitory activity for harmful algicides *(Microcystic aeruginos, Anabaena flos-aquae, Stephanodiscus hantzschii, Cyclotella, Peridinium bipes)*, while non-harmful algae *(Aulacoseira granulata* and *Scenedesmus actus)* were comparatively less

affected by these NQ derivatives. In particular, compounds **4c-6**, **4c-2**, **5c-6**, and **5c-2** were the most potent against 5 categories harmful algicidies with IC_{50} lower than 1μM. Especially, compounds **5c-2** was extremely competent and selective inhibitors against species with IC_{50} values ranging from 0.04 to 0.78μM. The while compounds **4k**, and **9k** were non-toxic to harmful algae *Microcystic aeruginos* and *Cyclotella* with the IC_{50} value ranging from 37.7 to 48.9μM. These results show that some NQ derivatives can potentially used as algicidies against harmful algal blooms.

I. INTRODUCTION

Algae are microscopic plants that do not have true leaves, stems, roots or flowers like other aquatic plants. They can be invisible to the naked eye or similar in size to other rooted aquatic plants. They form the base of the food chain in lakes and are eaten by a variety of organisms which are in turn eaten by larger insects, fishes and predators.

An algal bloom is a rapid increase or accumulation in the population of algae in an aquatic system. Blooms can be caused by several factors. When certain environment conditions such as warm weather, sunlight and excess levels of nutrients in the water help algae growth faster. Some of these blooms are harmless, but a bloom that produces toxins which are detrimental to plants and animals, it is known as harmful algae blooms (HABs). Algae blooms can change the water's appearance from slightly discoloured to resembling pea soup or thick paint. Blooms can also give water a bad odour or taste. Algae blooms can be change any colour such as brown, red, blue or green, but the red or green coloured blooms are most common referred to red or green tides.

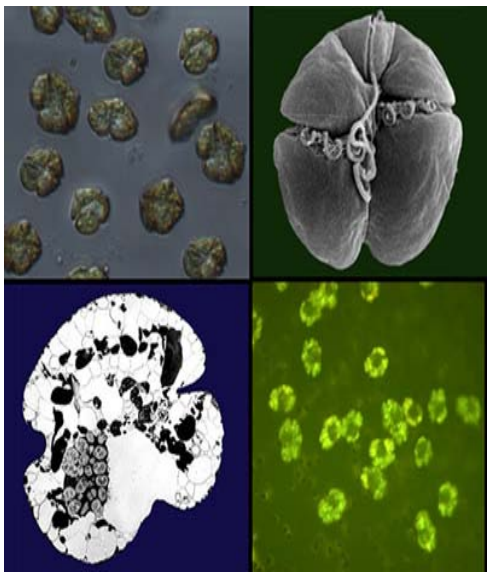


Fig. 1. Algal blooms can present problems for ecosystems and human society

Algal blooms occur in both saltwater and freshwater environments and cause harm through two primary mechanisms. The first category of impacts is the production of toxins. Toxins may kill fish or shellfish directly, or may cause one of several human illnesses following ingestion of contaminated seafood. The second category of impacts is high biomass accumulation, which, in turn, leads to environmental damage or degradation. These effects can include light attenuation, clogging of fish gills, or depletion of dissolved oxygen upon decay of the algal cells. Harmful algal blooms (HABs) have been increasing in prevalence for the past 30 years to the point where they occur along most of our coastlines and are a major problem in the word. Some HABs can even kill fish because of their physical shape, lodging in gill tissues and causing a physiological response leading to death.

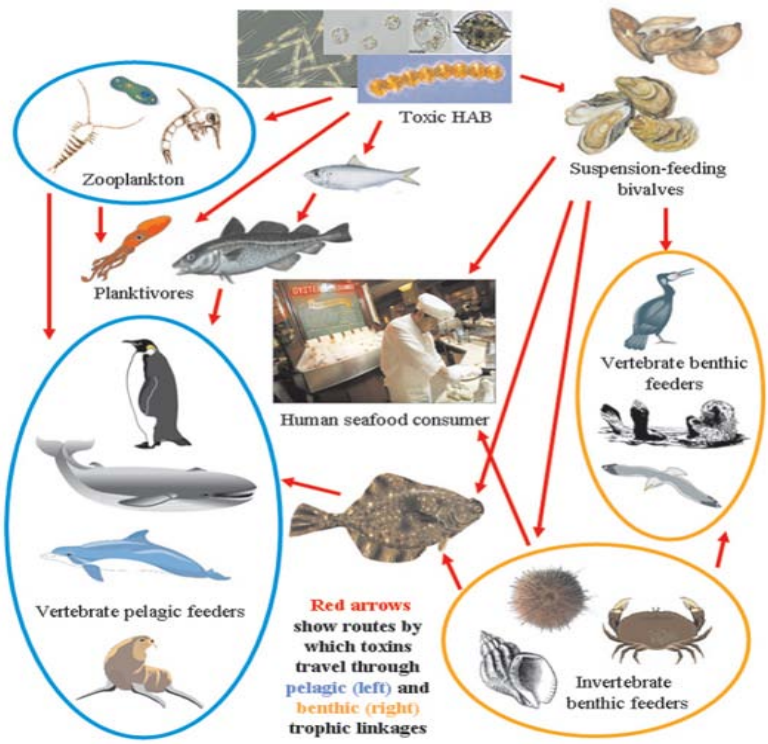


Fig. 2. Trophic linkages between HABs and their ecosystems

The impacts of blooms are felt in many ways such as human health is placed at risk, ecosystems are altered, marine mammals are injured or killed, and the fishing, aquaculture and recreation industries suffer economic losses. Moreover, dense growths of red tide algae cause

severe problems, such as hindering boat traffic, blocking approaches, obstructing wash processes and catching cavities, creating unattractive foul-smelling loads and killing natural biota [1, 2]. The growth of red tide algal inhabitants and increasing algal toxin levels are involved in causing severe diurnal instability in the dissolved oxygen levels, which can kill many aquatic animals [2, 3, 4]. Biotoxins from HABs are transferred throughout the food web when toxic algal cells are eaten by zooplankton, fish, and shellfish that are, in turn, eaten by other animals and humans. (G. Wikfors).

The challenge for the research community is to increase the understanding of harmful algal blooms and provide the tools to enable coastal communities and managers to reduce the impact of this problem. Over the past decade, there have been several reports dealing with how to better understand and respond to the harmful algal bloom issue. The seriousness of the problem has been examined in detail and a strong case for action has been established. Until now, many scientists have conducted physiological and ecological studies in the hope of reducing the extent of damage to fisheries caused by HABs [5, 6]. The approaches to direct bloom intervention can be grouped into three categories: mechanical, physical/chemical and biological control. Mechanical control involves the use of filters, pumps and barriers to remove or exclude HAB cells, dead fish or other bloom- related materials from impacted waters. Physical/chemical control involves the use of chemical or mineral compounds to kill, inhibit or remove HAB cells. Biological control involves the use of organisms or pathogens (e.g., viruses, bacteria, parasites, and shellfish) that can kill, lyse or remove HAB cells. The application of clay is one of physical/chemical control that can treat the environmental problems but the toxins released from flocculated cells and the adverse effects on other organisms need to be considered [7]. Clay flocculants are effective in the treatment of *Cochlodinium*, which causes fish deaths in a finfish cage culture in coastal Japan [8, 9] and in Chinese mariculture ponds [10]. Yellow loses is effective in sedimenting dinoflagellates [11, 12]. However, ruptured or damaged cells may release intracellular toxins into the surrounding water, which require the use of expensive removal processes, such as activated carbon and or oxidative ozone and chlorine [13]. Mechanical and physical/chemical methods have been devised in an attempt to manage HABs with limited success [14, 15].

The application of chemicals is one of the most common methods of controlling the development of noxious phytoplankton, but their use has limitations, such as toxicity towards

non-target species [16, 17]. The discovery and use of natural compounds that feature selective toxicity towards phytoplankton communities and which are nontoxic to other aquatic species has been significant advance in the management of aquatic ecosystems [35]. Therefore, considerable effort has been made to identify new compounds that are selectively effective against green tide algae.

Quinones are ubiquitous in nature and constitute an important class of naturally occurring compounds. They are found in plants, fungi, and bacteria [29]. These compounds are aromatic rings with two ketone substitutions [30]. Large number of quinones has been associated with antitumor, antibacterial, antimalarial and antifungal activities [31, 38]. From a toxicological perspective, quinones possess two principal chemical properties that confer their reactivity in biological systems. Quinones are oxidants and electrophiles, and the relative contribution of these properties to quinone toxicity is influenced by chemical structure, in particular substituent effects [36]. The antitumor activity is exhibited predominantly by three groups of naturally occurring quinines such as benzoquinone, naphthoquinone and anthraquinone.

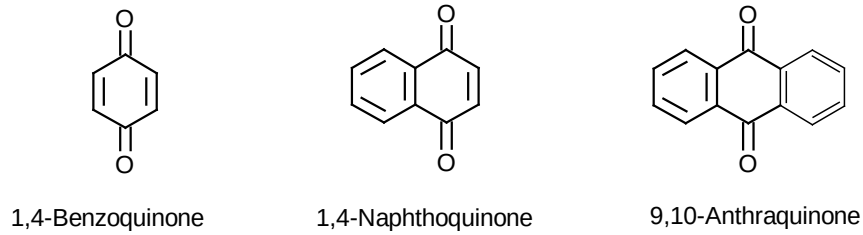
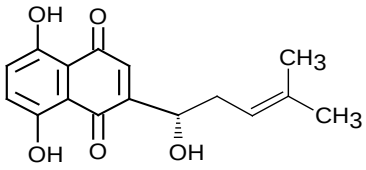


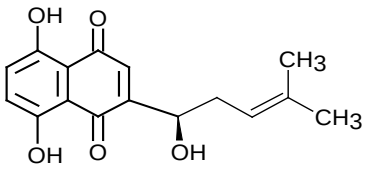
Fig. 3. Chemical structure of quinones

Mitomycin and streptonigrin possess *p*-benzoquinone moiety with hetercylic groups whereas anthraquinone, doxorubicin and daunorubicin consist of anthraquinone moiety. Some naphthaquinone antibiotics such as lapachol and lapinone are also found to be cutotoxic to tumor cell [32]. Nearly 300 naphthoquinones of different structural types have been isolated from plants, bacteria and fungi. These natural occurring compounds have long been used in folk medicine, and more recent studies have proved the therapeutic value of both natural synthetic naphthoquinones, particularly as antiparasitic and anticancer agents [33, 34].

In 1999, the enantiomeric naphthoquinone natural products Alkannin and Shikonin (A/S) are potent pharmaceutical substances with a wide spectrum of biological activity including antibacterial, anti-flammatory and comprise active ingredients for several pharmaceutical preparations. Alkannin and Shikonin are optical antipodes of plant origin, being mainly found in the roots of the pharmaceutical plants *Alkannatinctoria* and Lithospermum erythrorhizon. It also was found to have good cytotoxicity against L1210 cells. Moreover, the previous study Shikonin derivatives with a naphthoquinone moiety were designed and synthesized in order to investigate the cytotoxicity in vitro and antitumor activity in mice bearing sarcoma 180.



Alkannin



Shikonin

Fig. 4. Chemical structures of Alkannin and shikonin

In 2005, a number of processes of developing selective control techniques for aquatic herbicides have been evaluated [18]. Some chemicals have been used to mitigate HABs, but a search for safer and selective algicidal agents is needed to better control HABs. Copper sulphate, chelated copper compounds, and diuron (3-[3,4-dichlorophenyl]-1,1-dimethylurea), etc. are currently approved by U.S. Environmental Protection Agency [19]. The more direct control method involves the use of chemical treatments, such as algicides, including copper, reglone a (diquat, 1,1-ethylene-2,2-dipyridilium dibromide), potassium permanganate, chlorine and simazine (2-chloro-4,6-bis(ethylamino)-s-triazine, clotrimazole [19, 20, 21]. Unfortunately, these compounds have undesirable characteristics, including broad-spectrum toxicity towards phytoplankton, subsequent water quality deterioration, and lengthy persistence that creates environmental safety concerns [19, 22]. Natural antialgal compounds extracted from a range of bio resources have also reported. One of these compounds is 9,10-anthraquinone, which is found in plant tannin extract [27] has a high degree of selective toxicity towards *cyanobacterium*.

(a) (b)

Fig. 5. Structure of 2-[methylamino-N-(1'-methyl-4'-N,N-dimethylaminobutyl)] anthraquinone diphosphate (a) and 2-[methylamino-N-(1'-methylethyl)]-9,10-anthraquinone diphosphate (b)

By use of microtiter plate bioassays, a novel group of compounds (Fig. 5) derived from the natural 9,10-Anthraquinone have been found to be much more selective toxicity towards cyanobacteria than other phytoplankton.

In comparison with copper-based products and diruon, anthraquinone-59 (b) offers greater selective toxicity towards cyanobacteria than other phytoplankton. Anthraquinone-59 is much more selective O.perornata than the preferred types of phytoplankton, such as green algae. Another potential advantage of anthraquinone-59 involves the public's negative perception of the use of herbicides such as diuron in food fish production ponds. Anthraquinone-59 is derived from the natural compounds 9,10-anthraquinone, which is found in certain plants [27]. Also, anthraquinone-59 has much lower persistence in pond water (half-life of 19h) than diuron, which can persist for weeks in the water column after application to catfish aquaculture ponds (half-life of 2 weeks in pond water). Enviromental safety issues also persist on the use of copper sulphate in catfish ponds, since the copper accumulates in the pond sediments and long-term applications may adversely affect microbial activity in the pond sediments [28]. These include furano-diterpenses [23] at low biosurfactant concentrations [24], alleo-chemical [25], and barley straw. Other studies [25, 26] have also attempted to manage red tide growth using controlling agents.

Naphthoquinones are widely distributed in nature and have been used for centuries in home remedies as well as in cosmetics. Many clinically important antitumor drugs and

algicides containing quinone nucleus, such as mitoxantrones and saintopin, show excellent anticancer activity. These anticancer agents are effective inhibitors of DNA topoisomerase, and it is understood that the cytotoxicity of 1,4-naphthoquinone analogues results from the inhibition of DNA topoisomerase. Furthermore, they can induce the formation of the semi-quinone radical, which can transfer an electron to oxygen to produce superoxide. In addition, a number of 1,4-naphthoquinone derivatives have been found to possess powerful pharmacological effects such as antibacterial, antifungal, anti-inflammatory, anti-thrombotic, antiplatelet, antiviral, antiallergic, apoptotic, lipoxygenase inhibiting, radical scavenging, and antiringworm activities [37]. The biological activity imparted by 1,4-naphthoquinones in most cases relies upon their ability to accept one or two electrons to form radical anion and the redox property which is further responsible for compounds to catalytically cycle and generate oxidative radicals, such as hydrogen peroxide and superoxide which damage the cell. Many amino and heterocyclic 1,4-naphthoquinones have been used for the construction of numerous biological profile resulting from the presence of heteroatom, sulphur or nitrogen atoms at the position in the side chain or inside the ring.

Until now, there have been a few reports on naphthoquinone derivatives, exhibiting potent biological properties including antimalarial activity as well as antibacterial and antiparasitic properties. However, the inhibitory activity for harmful algicides *(Microcystic aeruginos , Anabaena flos-aquae, Stephanodiscus hantzschii, Cyclotella, Peridinium bipes)* of naphthoquinone derivatives have not been reported to the best of our knowledge. Therefore, the design of environmentally safe, selective algicides to manage the growth of harmful algal species has also been an ongoing research topic. In this preliminary SAR study, 2- or 6-substitued 5,8-dimethoxy-1,4-naphthoquinone (DMNQ) derivatives were synthesized and the effect of these derivatives on the growth of a number of harmful algal species were tested. This thesis describes the results of laboratory tests on the efficacy of the synthetic NQ derivatives to control harmful algal species. The NQ derivatives were very competent and selective against the HABs studied and exhibited IC_{50} in the nanomolar range.

II. MATERIALS AND METHODS

1. Materials

The chemical and reagents used in this work were obtained from Sigma-Aldrich or Merck. All the moisture-sensitive reactions were performed in an inert atmosphere with either N_2 using distilled dry solvents.

^{1}H NMR spectra in each compound were acquired with spectrometer at 296 K, in 300 MHz. Each sample was dissolved in $CDCl_3$ or DMSO using TMS (tetramethylsilance) as internal standard. Chemical shifts (δ scale) are quoted in parts per million (ppm) and the following abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiple) some combinations of these were made by DEPT editing of the spectra. J coupling constants were measured in Hz (Hertz) unit.

Column chromatography was performed by using silica gel after preparing a slurry with eluent mixtureand packing it into the chromatography column. A fraction of the collected samples were analyzed by thin layer chromatography (TLC).

2. Algal cultures, mediums and culture conditions

The *Microcystic aeruginos , Anabaena flos-aquae, Stephanodiscus hantzschii, Cyclotella, Peridinium bipes, Aulacoseira granulat, Scenedesmus actus* were kindly provided by Professor. M-S. Han from life science department of Hanyang University in Korea.

Cultures of *Microcystic aeruginos , Anabaena flos-aquae, Stephanodiscus hantzschii, Cyclotella and Peridinium bipes*, and non-harmful algae were grown in culture flask (Becton Dickinson Labware, Franklin Lakes, New Jersey, USA) at 20^{o}C under constant light in Guillard's f/2 medium with filtered seawater, as reported previously [27]. The f/2 medium was prepared by sterile-filtering sea water with 0.22 μm filtration units (Nalgene, Rochester, New

York, USA) and enriched aseptically using nutrients and vitamins purchased from Sigma (St.Louis, MO, USA).

3. Screening of algicidal activities of NQ compounds

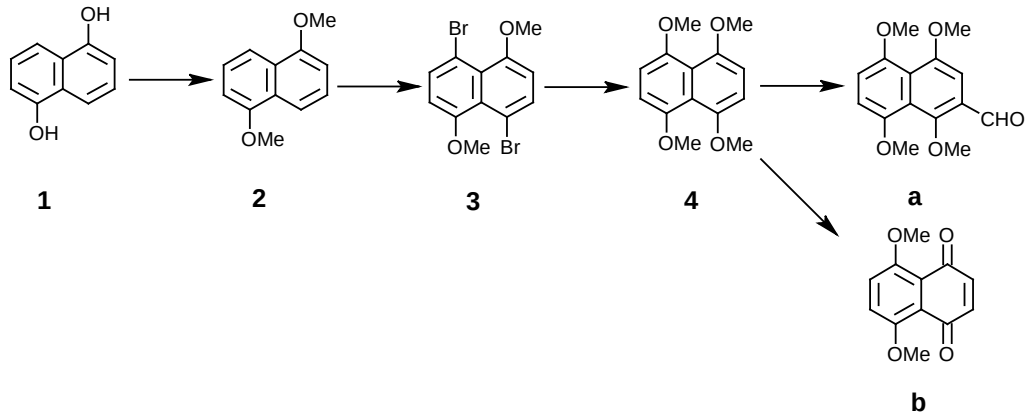
The algicidal activity of the different NQ compounds against Microcystic aeruginos , Anabaena flos-aquae, Stephanodiscus hantzschii, Cyclotella, and Peridinium bipes, were examined at various concentrations. Each experiment was carried out in 24 well tissue culture test plates (SPL) with approximately one ml total volume per well. Various concentrations of the test compounds were introduced to the cultures during the exponential growth phase. All the microalgae were exposed to the compounds at final concentrations of 100, 50, 20, 10, 5, 2, 1 μM and 0.05 μM. As the non-hamrful algal control, the NQ compounds were applied at concentrations > 500 μM. The control cultures were performed without the NQ compounds. The algal cell density was counted 3 days after inoculation them with the compounds. The algal cells were counted using a Burker Turk hemacytometer with Sedgwick-Rafter counting chamber under an Olympus light microscope with x40 and x100 magnification (Olympus Co., Tokyo, Japan). Algicidal activity profiling of the NQ compounds growth rates were then calculated and are expressed as the reduction ratio (%) was determined using the following equation : % Algicidal activity = (1-*Tt/Ct*) x 100, where *T* (treatment) and *C* (control) are the cell densities with and without each NQ compound at different concentrations and *t* is the inoculation time (day).

4. Statistical data analysis

The experiments were carried out at least three times. The data is reported as the mean ± SD. All statistical analyses were performed using the SPSS 17.0 software (SPSS, USA). The statistical significance of the differences between the mean values was determined by on-way variance analysis (ANOVA) followed by a Turkey's HSD post hoc test. A p value < 0.05 was considered signific.

5. Methods

Scheme I



5.1. Procedure for the synthesis of compounds of scheme I

5.1.1. 1,5-Dimethoxynaphthelene (2)

Under ice cooling, dimethyl sufate (156 g, 1.24 mol) was dropwise to a solution of 1,4-dihydroxynaphthalene (1) (100 g, 0.62 mol) in 10% aqueous NaOH (500 ml), and stirred for 2h. The precipitate was collected by filtration, washed with 5% aqueous NaOH (200 ml x 2), and then dried in oven. The crude product was recrystallized from benzene to give compound (b) (73.0 g, 63%) as white solid: m.p 181-182^{o}C.

^{1}H-NMR ($CDCl_3$, 400 Hz) δ 7.70 (d, J=8.8 Hz, 2H), 7.38 (t, J=8.0 Hz, 2H), 6.98 (d, J=8.0 Hz, 2H), 3.94 (s, 6H).

5.1.2. 4,8-Dibromo-1,5-dimethoxynapthalene (3)

To a solution of 1,5-Dimethoxynaphthelene (2) (10.0 g, 0.053 mol) in acetonitrile (160 ml) was dropwise solution of N-bromosuccinimide (21.0 g, 0.118 mol) in acetonitrile (180 ml). The resulting mixture was stirred at room temperature under nitrogen for 2.5h. The solid was collected filtration, washed with acetronitrile (50 ml x 2) and with 20 ml of hexane to give compound (c) (12.7 g, 69%) as a white solid: m.p 187-188^{o}C.

^{1}H-NMR ($CDCl_3$, 400 Hz), δ 7.86 (d, J=8.4 Hz, 2H), 6.72 (d, J=8.4 Hz, 2H), 3.91 (s, 6H).

5.1.3. 1,4,5,8 Tetramethoxynaphthalene (4)

To a solution of 4,8-Dibromo-1,5-dimethoxynapthalene (3) (14.5 g, 0.04 mol) in N,N-dimethylformamide (300 ml), and MeOH (300 ml) was added copper iodide (26.6 g, 0.14 mol). The resulting mixture was reflux for 30h. 500 ml of ice water was added to the solution. The mixture was filtered, washed with water (100 ml), and dried in oven, separated with chloroform. The crude product was recrystallized from benzene to give compound (d) (6.5 g, 62.5%) as a white solid: m.p 168-169^{o}C.

^{1}H-NMR ($CDCl_3$, 400 Hz), δ 6.85 (s, 2H), 3.90 (s, 12H).

5.1.4. 2-formyl-1,4,5,8-tetramethoxynaphthalene (a)

To solution of DMF 8.15 ml (0.1 mol) and $POCl_3$ 9.87 ml (0.1 mol) in 250 ml round flask. The solution of 1,4,5,8 Tetramethoxynaphthalene (4) (5.1 g, 0.02 mmol) in chloroform (45 ml) was added dropwise in flask. The resulting mixture was stirred at 200^{o}C for 6h, after cooling the mixture was added ice water and extracted with methylene chloride and washed with water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was recrystallized from ethyl acetate and n-hexan to afford 5.3 g (Yield: 96%) as a yellow solid: m.p 127-128^{o}C.

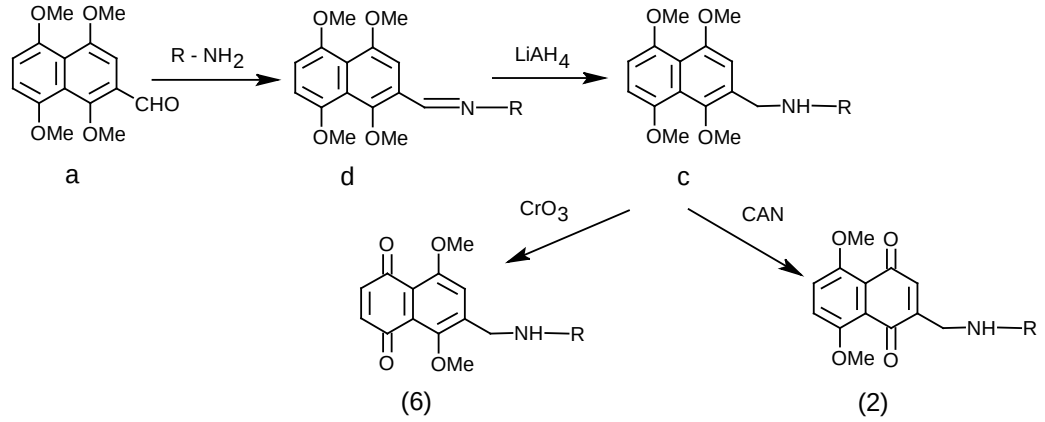
^{1}H-NMR ($CDCl_3$, 300 Hz), δ 10.6 (1H, s), 7.20 (1H, s), 6.93 (1H, d, J=8.7 Hz), 6.77 (1H, d, J=8.7 Hz), 4.0 (3H, s), 3.99 (3H, s), 3.92 (3H, s), 3.91 (3H, s)

5.1.5. 5,8-Dimethoxynaphthalene-1,4-dione (b)

To a solution of 1,4,5,8 Tetramethoxynaphthalene (4) (10 g, 40.3 mmol) in acetronitrile (450 ml) and chloroform (150 ml) was added dropwise a solution of cerium ammonium nitrate (54 g, 98.5 mmol) in water (300 ml). The resulting mixture was stirred at room temperature for 1h, after solution was added water (600 ml) and $CHCL_3$ (600 ml). The organic layer was separated, dried over sodium sulphate, and concentrated in vacuo. The residue was recrystallized from MeOH to give compound (f) (4.80 g, 54.6%) as a red solid: m.p 122-123^oC.

^{1}H-NMR ($CDCl_3$, 400 Hz) δ 7.33 (s, 2H), 6.79 (s, 2H), 3.97 (s, 6H).

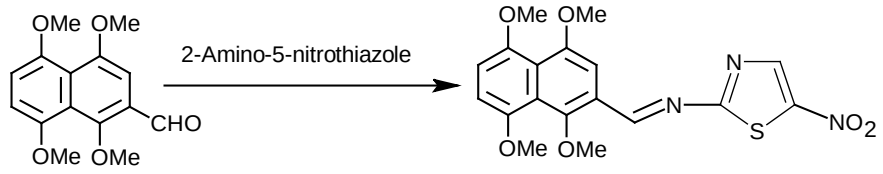
Scheme II



5.2. Procedure for the synthesis of compounds of scheme II

5.2.1. General produce for the synthesis of compounds (1d-5d)

1d: (5-nitro-thiazol-2-yl)-(1,4,5,8-tetramethoxy-naphthalen-2-ylmethylene)-amine

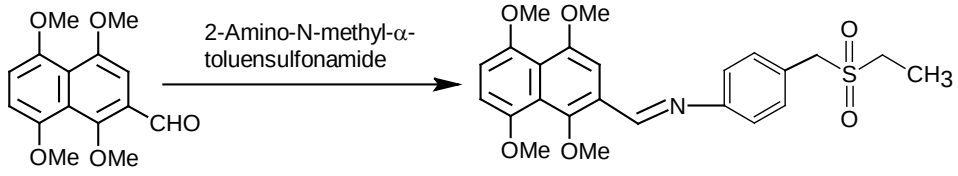


1d

To a 100 mL round flask fitted with a Dean-Stark trap and a condenser were added benzene 20 mL, 2-Formyl-1,4,5,8-tetramethoxynaphthalene (2 g, 7.25 mmol), 2-Amino-5-nitrothiazole (1.16 g, 7.25 mmol), triethylamine (1.03 mL, 7.25 mmol), and acetic acid (300 µl, pH 4-5), the mixture was refluxed for 20h and then the water removed by azeotropic distillation. After cooling to room temperature, the reaction mixture was washed successively with 5% HCl, saturated $NaHCO_3$, 5% acetic acid and water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was recrystallized from ethyl acetate and hexan to afford 2.78 g (Yield: 87.9%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.0 (1H, s), 8.5 (1H, s), 7.5 (1H, s), 6.1 (1H, d, J=9.3 Hz), 5.9 (1H, d, J=9.3 Hz), 4.7 (2H, d, J=5.3 Hz), 4.0 (12H, s).

2d: (5-nitro-thiazol-2-yl)-(1,4,5,8-tetramethoxy-naphthalen-2-ylmethyl)-amine

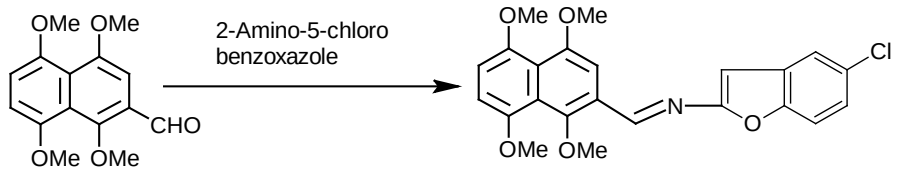


2d

The resulting residue was recrystallized from ethyl acetate and hexan to afford 3 g (Yield: 87%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.0 (1H, s), 7.6 (1H, s), 7.5 (2H, d, J=9.3 Hz), 7.3 (1H, s), 7.0 (2H, d, J=9.3 Hz) 4.3 (1H, s), 4.0 (12H, s), 2.7 (3H, d, J=6).

3d: (5-chloro-benzooxazol-2-yl)-(1,4,5,8-tetramethoxy-naphthalen-2-ylmethylene)-amine

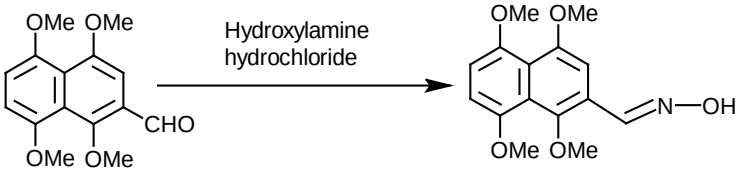


3d

The resulting residue was recrystallized from ethyl acetate and hexan to afford 2.78 g (Yield: 89.9%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.9 (1H, s), 7.7 (1H, d, J=9.6 Hz), 7.65 (1H, s), 7.45 (1H, s), 7.3 (1H, d, J=9.6 Hz), 7.1 (1H, d, J=10 Hz), 6.9 (1H, d, J=10 Hz), 4.0 (12H, s).

4d: ***2-(hydroxyiminomethyl)-1,4,5,8-tetramethoxynaphthalene***



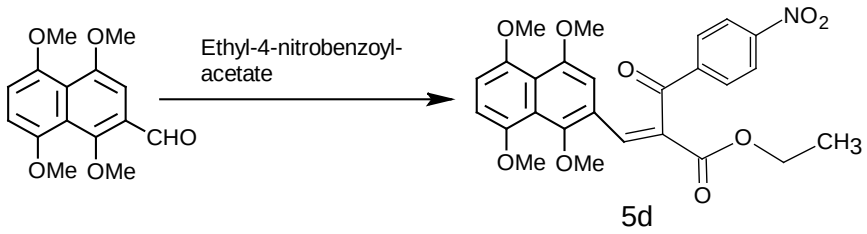
4d

To a 100mL round bottom flask was added 2- Formyl- 1, 4, 5, 8-tetramethoxynaphthalene (5.52 g, 20 mmol), $NH_2OH.HCl$ (1.67 g, 24 mmol) and ethyl alcohol and water (1:1) 100ml. The reaction mixture was stirred 2h at room temperature. After checking TLC, the mixture was extracted with 800 mL water and 500 mL ethyl ether 3 times. Separation of this compound by column chromatography on silicagel (ethyl acetate: n-hexan=1:4). Yield: 88% (5.31g).

Mp: 145 - 146^{o}C

^{1}H-NMR (300Hz, DMSO) δ 8.65 (1H, s), 7.90 (1H, s), 7.21 (1H, s), 6.92 (1H, d, J=8.7Hz), 6.92 (1H, d, J=8.7Hz), 6.88 (1H, d, J=8.7Hz), 3.96 (6H, s), 3.91 (3H, s), 3.79 (1H, s).

5d: ***Ethyl 2-(4-nitrobenzol)-3-(1,4,5,8-tetra-methoxy-2-naphthyl)-2-propenoate***



5d

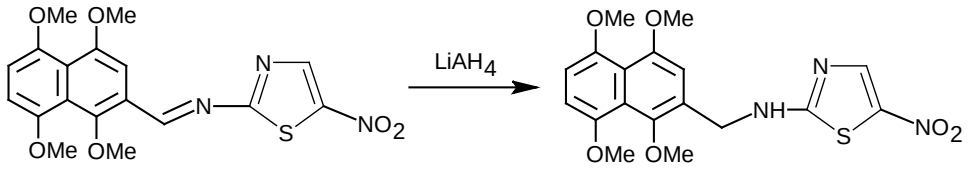
The resulting residue was recrystallized from ethyl acetate and ether to afford 5.3g (89%).

M.p: 116 - 118^{o}C

^{1}H-NMR (200 MHz, DMSO) δ 8.55 (1H, t), 8.24 (2H, d, J=9.0), 8.10 (2H, d, J=9.0 Hz), 6.87 (2H, s), 6.54 (1H, s), 4.28 (2H, q, J=7.1 Hz), 3.93 (3H, s), 3.82 (6H, s), 3.58 (3H, s), 1.23 (3H, t, J=7.1Hz).

5.2.2. General produce for the synthesis of compounds (1c-4c)

1c: (5-Nitro-thiazol-2-yl)-(1,4,5,8-tetramethoxy-naphthalen-2-ylmethyl)-amine



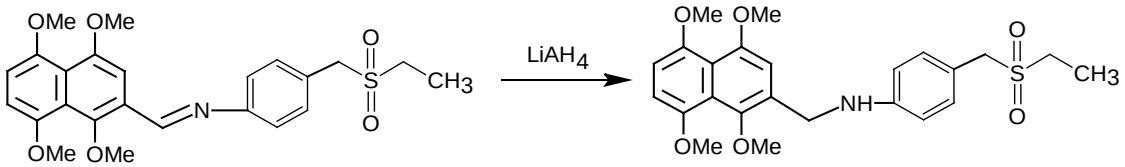
1d 1c

To a stirred solution of *(5-nitro-thiazol-2-yl)-(1,4,5,8-tetramethoxy-naphthalen-2-ylmethylene)-amine* **(1d)** (1.97 g, 4.90 mmol) in 30 mL of tetrahydrofuran at room temperature was slowly added $LiAlH_4$ (195.7 mg, 4.90 mmol) over a period of 10 min. The mixture was then stirred at room temperature for 30 min. The reaction mixture was extracted with methylene chloride and washed with water. The organic layer was dried over $MgSO_4$ and

concentrated under reduced pressure. The resulting residue was recrystallized from ethyl ether to afford 1.86 g (Yield: 77.4%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.5 (1H, s), 7.7 (1H, s), 6.8 (2H, s), 4.7 (2H, d, J=5.3 Hz), 4.0 (6H, s).

2c: N-methyl-C-{4-[1,4,5,8-tetramethoxy-naphthalen-2-ylmethyl)-amino]-phenyl}-methanesulfonamide

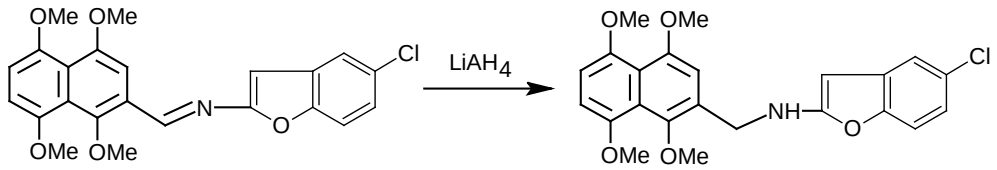


2d 2c

The resulting residue was recrystallized from ethyl ether to afford 1.68 g (Yield: 75%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.0 (1H, s), 7.6 (1H, s), 7.5 (2H, d, J=9.3 Hz), 7.3 (1H, s), 7.0 (2H, d, J=9.3 Hz) 4.5 (2H, d, J=6.8), 4.3 (1H, s), 4.0 (12H, s), 2.7 (3H, d, J=6).

3c: (5-chloro-benzooxazol-2-yl)-(1,4,5,8-tetramethoxy-naphthalen-2-ylmethyl)-amine

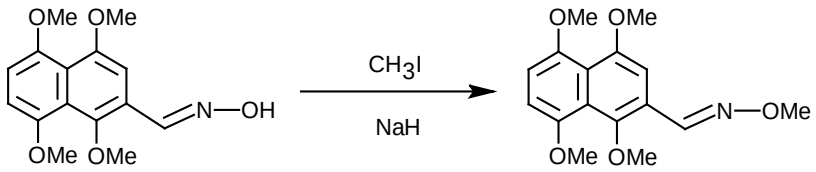


3d 3c

The resulting residue was recrystallized from ethyl ether to afford 1.75 g (Yield: 83.7%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.3 (1H, d, J=9.6 Hz), 7.15 (1H, s), 7.0 (1H, s), 6.9 (1H, d, J=9.6 Hz), 7.1 (1H, d, J=10 Hz), 6.9 (2H, d, J=10 Hz), 5.7 (1H, t, J=6.8 Hz), 4.8 (2H, d, J=6.8 Hz), 4.0 (12H, s).

4c: 2-(Methoxyiminomethyl)-1,4,5,8-tetramethoxynaphthalene



4d 4c

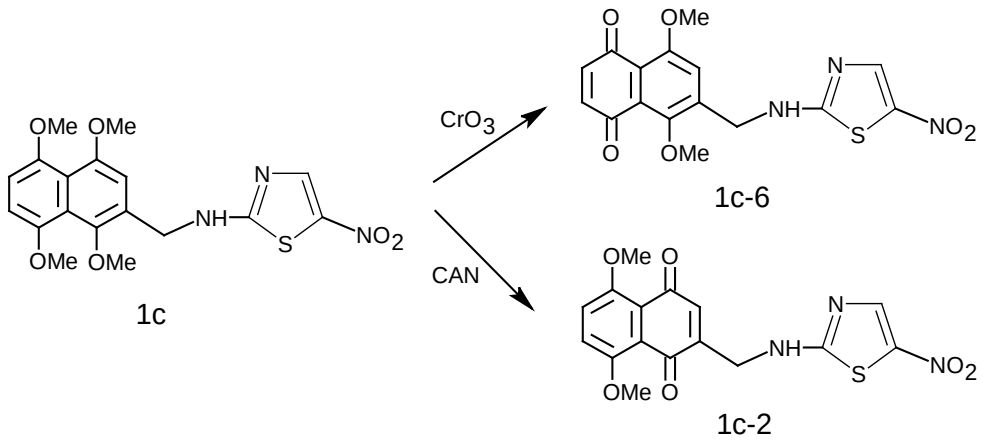
To a 100 mL round bottom flask was added 2-(hydroxyiminomethyl)-1,4,5,8-tetramethoxynaphthalene (528 mg, 2 mmol), THF 10 mL at room temperature. The mixture was stirred and added slowly NaH (62 mg, 2.6 mmol) during 10 minutes. And then, the reaction mixture was added methyl iodide (162 µl, 2.6 mmol) and stirred about 6h. After checking TLC, this reaction mixture was extracted with 50 mL water and methylene chloride 50 mL 2 times. Separation of this compound by column chromatography on silicagel (ethyl acetate: n-hexan=1:4). Yield: 90% (550 mg).

Mp: 114 - 116^{o}C

^{1}H-NMR (300Hz, DMSO) δ 8.84 (1H, s), 7.290(1H, s), 6.90 (1H, d, J=8.7Hz), 6.86 (1H, d, J=8.7Hz, 4.03 (3H, s), 3.99 (3H, s), 3.95 (3H, s), 3.90 (3H, s), 3.76 (3H, s).

5.2.3. General produce for the synthesis of compounds (6) and (2)

1-6 & 1-2



1c-6: 5,8-Dimethoxy-6-[(5-nitro-thiazol-2-ylamino)-methyl]-[1,4]naphthaquinone

To a stirred solution of *(5-Nitro-thiazol-2-yl)-(1,4,5,8-tetramethoxy-naphthalen-2-ylmethyl)-amine* (1c) (786.74 mg, 1.95 mmol) in acetone (20 mL), at room temperature were added H_2SO_4 160 µl, H_2O 4mL, CrO_3 203.2 mg (1.95 mmol). The mixture was the stirred at room temperature for 1h. The reaction mixture extracted with methylene chloride and washed with water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was recrystallized from ethyl acetate and n-hexan. Yield: 80.8% (635.6mg).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.5 (1H, s), 7.7 (1H, s), 6.8 (2H, s), 4.7 (2H, d, J=5.3 Hz), 4.0 (6H, s).

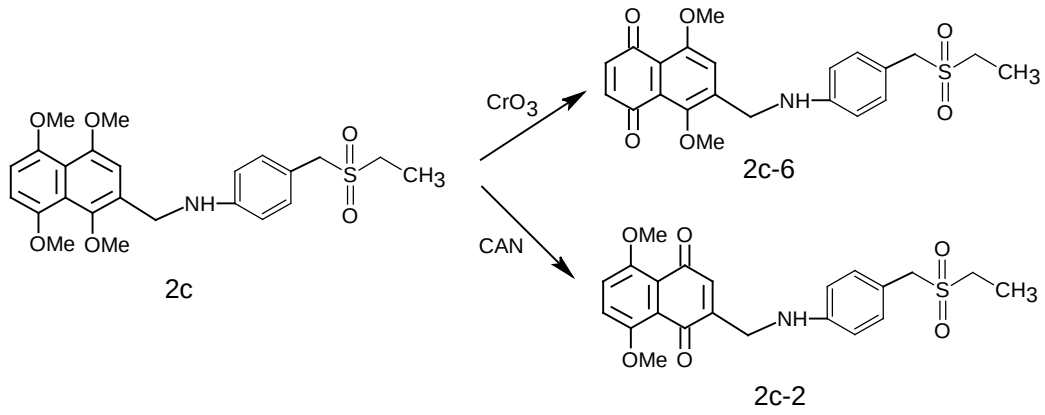
1c-2: 5,8-Dimethoxy-2-[(5-nitro-thiazol-2-ylamino)-methyl]-[1,4]naphthaquinone

To a stirred solution of *(5-Nitro-thiazol-2-yl)-(1,4,5,8-tetramethoxy-naphthalen-2-ylmethyl)-amine* (1c) (786.74 mg, 1.95 mmol) in acetonitrile (15 mL) at 5^{o}C temperature were added a solution of ammonium cerium (IV) nitrate (2.72 g, 4.87 mmol) in 3.5 mL of water. The mixture was then stirred at room temperature for 1h. The reaction mixture extracted with methylene chloride and washed with water. The organic layer was dried over $MgSO_4$ and

concentrated under reduced pressure. The resulting residue was recrystallized from ethyl acetate and n-hexan. Yield: 72.1% (560.3 mg).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.5 (1H, s), 8.5 (1H, s), 7.5 (1H, s), 6.1 (1H, d, J=9.3 Hz), 5.9 (1H, d, J=9.3 Hz), 4.0 (12H, s).

2c-6 & 2c-2



2c-6: C-{4-[(1,4-Dimethixy-5,8-dioxo-5,8-dihydro-naphthalen-2-ylmethyl)-amino]-phenyl}-N-methyl-methanesulfonamide

The resulting residue was recrystallized from ethyl acetate and n-hexan. Yield: 80% (717.6 mg).

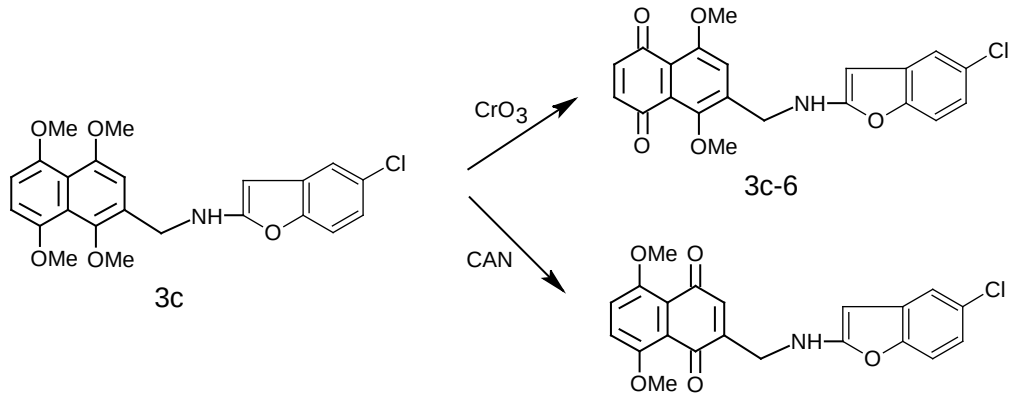
^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.5 (1H, s), 7.3 (2H, d, J=9.3 Hz), 6.9 (2H, d, J=10.6 Hz), 6.6 (2H, d, J=10.6 Hz), 4.5 (2H, d), 4.0 (6H, s), 2.6 (3H, d, J=10.5 Hz).

2c-2: C-{4-[(5,8-Dimethixy-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl)-amino]-phenyl}-N-methyl-methanesulfonamide

The resulting residue was recrystallized from ethyl acetate and n-hexan. Yield: 70% (627.9 mg).

^{1}H-NMR (300 MHz, DMSO) δ 7.5 (1H, s), 7.6 (1H, s), 7.1 (2H, d, J=9.3 Hz), 6.7 (1H, d), 6.6 (2H, d, J=10.6 Hz), 4.3 (2H, d), 4.0 (6H, s), 2.6 (3H, d, J=10.5 Hz).

3c-6 & 3c-2



3c-6: 6-[(5-Chloro-benzooxazol-2-ylamino)-methyl]-5,8-dimethoxy-[1,4] naphthoquinone

The separation of this compound by column chromatography on silicagel (ethyl acetate: n-hexan=1:4). Yield: 87.6% (732.5 mg).

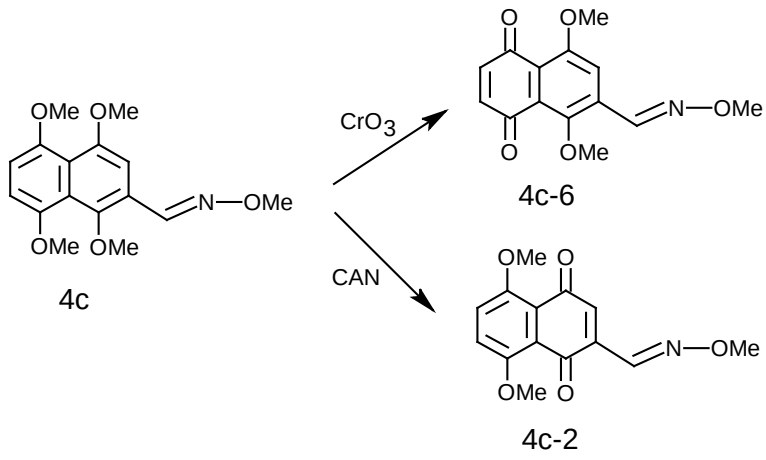
^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.3 (1H, d, J=9.6 Hz), 7.15 (1H, s), 7.0 (1H, s), 6.9 (1H, d, J=9.6 Hz), 7.1 (1H, d, J=10 Hz), 6.9 (2H, d, J=10 Hz), 5.7 (1H, t, J=6.8 Hz), 4.8 (2H, d, J=6.8 Hz), 4.0 (6H, s).

3c-2: 2-[(5-Chloro-benzooxazol-2-ylamino)-methyl]-5,8-dimethoxy-[1,4] naphthoquinone

The separation of this compound by column chromatography on silicagel (ethyl acetate: n-hexan=1:4). Yield: 52.9% (442.24 mg).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.3 (1H, d, J=9.6 Hz), 7.15 (1H, s), 7.0 (1H, s), 6.9 (1H, d, J=9.6 Hz), 7.1 (1H, d, J=10 Hz), 6.9 (2H, d, J=10 Hz), 5.7 (1H, t, J=6.8 Hz), 4.8 (2H, d, J=6.8 Hz), 4.0 (6H, s).

4c-6 & 4c-2



4c-6: 2-(Methoxyiminomethyl)-1,4-dimethoxy-5,8-dihydro-5,8-naphthalenedione

The separation of this compound by column chromatography on silicagel (ethyl acetate: n-hexan=1:2). Yield: 71% (196.3 mg).

M.p: 173 - 174^{o}C

^{1}H-NMR (300Hz, $CDCl_3$) δ 8.23 (1H, s), 7.80 (1H, s), 6.84 (1H, d, J=10.2 Hz), 6.79 (1H, d, J=10.2 Hz), 4.06 (3H, s), 4.03 (3H, s), 3.84 (3H, s).

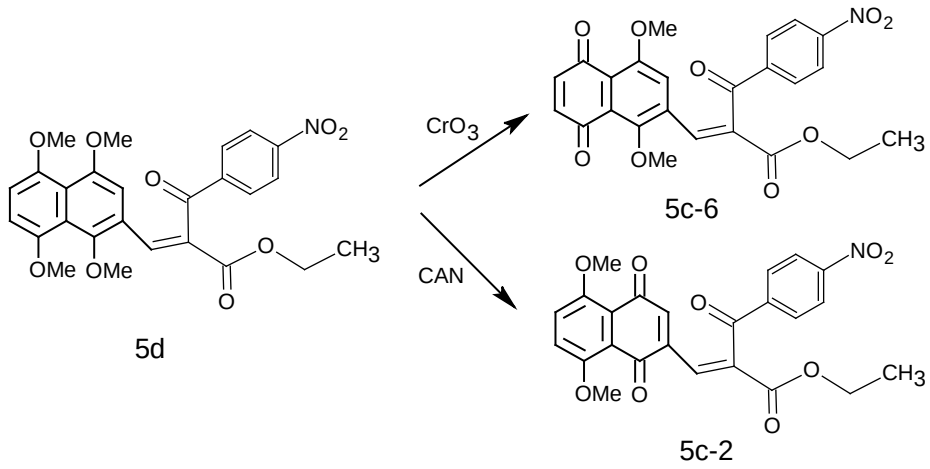
4c-2: 2-(Methoxyiminomethyl)-5,8-dimethoxy-1,4-dihydro-1,4-naphthalenedione

The separation of this compound by column chromatography on silicagel (ethyl acetate: n-hexan=1:4). Yield: 22% (60.8 mg).

M.p: 122 - 124°C

^{1}H-NMR (300Hz, $CDCl_3$) δ 8.26 (1H, s), 7.34 (2H, s), 7.20 (1H, s), 4.02 (3H, s), 4.03 (3H, s), 3.97 (6H, s).

5c-6 & 5c-2



5c-6: Ethyl 2-(4-nitrobenzol)-3-(1,4-dimethoxy-5,8-dioxo-5,8-dihydro-2-naphthalenyl)-2-propenoate

The separation of this compound by column chromatography on silicagel (ethyl acetate: n-hexan=1:4). Yield: 63% (587 mg).

M.p: 135 - 137°C

^{1}H-NMR (200 MHz, DMSO) δ 8.29 (2H, d, J=8.8 Hz), 8.28 (1H, s), 8.05 (2H, d, J=8.5 Hz), 7.20 (1H, s), 6.77 (2H, s), 4.30 (2H, q , J=7.1 Hz), 3.86 (3H, s), 3.74 (3H, s), 1.23 (3H, s).

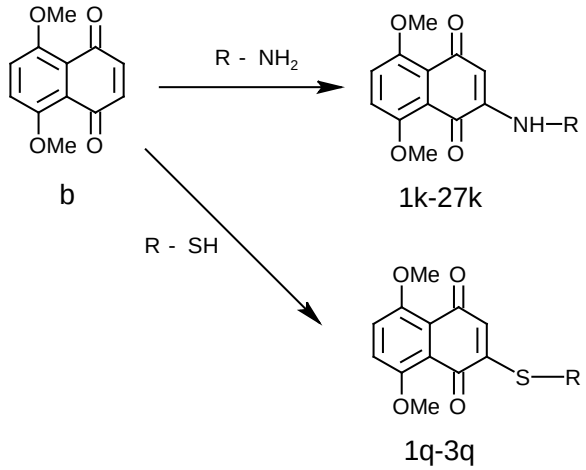
5c-2: Ethyl 2-(4-nitrobenzol)-3-(5,8-dimethoxy-1,4-dioxo-1,4-dihydro-2-naphthalenyl)-2-propenoate

The separation of this compound by column chromatography on silicagel (ethyl acetate: n-hexan=1:4). Yield: 31% (289 mg).

M.p: 158 - 160^{o}C

^{1}H-NMR (200 MHz, DMSO) δ 8.29 (2H, m), 8.08 (2H, m), 7.96 (1H, s), 7.30 (2H, m), 6.74 (1H, s), 4.26 (2H, q , J=7.1 Hz), 3.92 (3H, s), 3.89 (3H, s), 1.21 (3H, t, J=7.1 Hz).

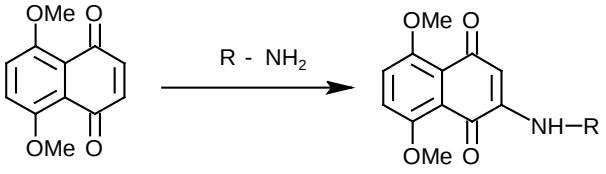
Scheme III



5.3. Procedure for the synthesis of compounds of scheme III

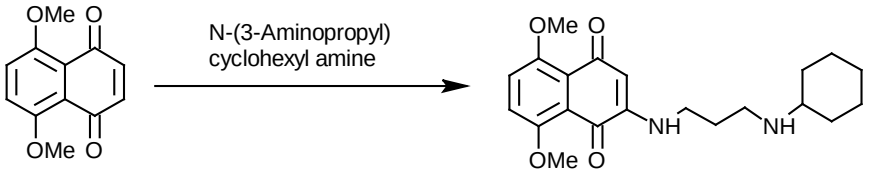
5.3.1 General procedure for the synthesis of compounds (1k-27k)

To a solution of 5,8-Dimethoxynaphthalene-1,4-dione (f) (301 mg, 1.38 mmol) in MeOH (30 ml) were added corresponding alkyl amine (2.07 mmol). The mixture was stirred at room temperature for 4h and evaporated under pressure. The crude product was purified by column chromatography (hexanes:EtOAc=2:1) to give the titled compounds **1k-27k.**



b 1k-27k

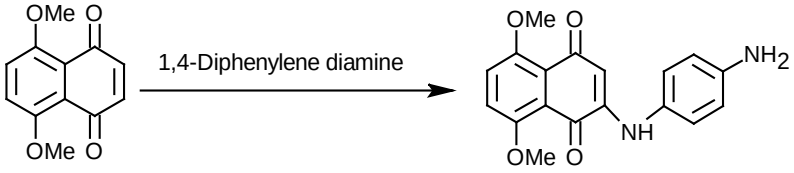
1k: **2-(3-Cyclohexylamino-propylamino)-5,8-dimethoxy-[1,4]naphthoquinone**



To solution of 5,8-Dimethoxynaphthalene-1,4-dione (301mg, 1.38mmol) in MeOH (30mL) were added N-(3-Aminopropyl cyclohexyl amine) (351.6µl, 2.07mmol). The mixture was stirred at room temperature for 4h and evaporated under reduced pressure. The crude product was purified by column chromatography (hexanes:EtOAc=1:4) to give the product. Yield: 52.5% (270 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.2 (2H, d, J=8 Hz), 5.8 (1H, s), 4.0 (6H, s), 3.4 (2H, m), 1.8 (2H, m), 1.5 (11H, m), 1.0 (2H, t, J=9.8 Hz).

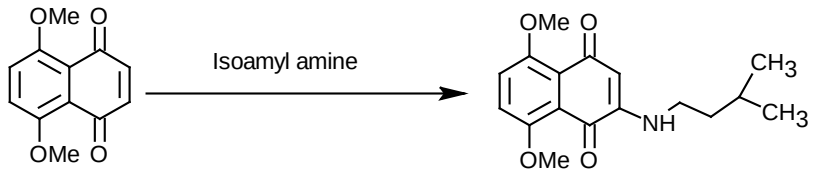
2k: 2-(4-Amino-phenylamino)-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc=1:2) to give the product. Yield: 53.2% (233 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.4 (1H, d, J=10.6 Hz), 7.35 (1H, d, J=10.6 Hz), 7.0 (1H, d, J=9.7 Hz), 6.7 (1H, d, J=9.7 Hz), 6.1 (1H, s), 5.4 (1H, s), 4.0 (6H, s).

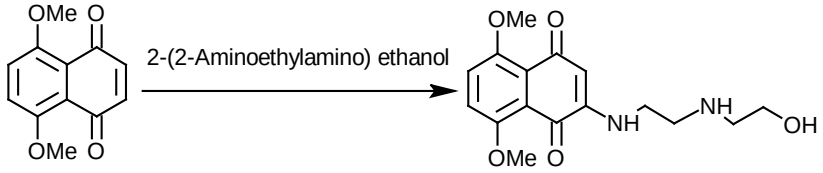
3k:5,8-Dimethoxy-2-(3-methyl-butylamino)-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc=1:2) to give the product. Yield: 67.7% (283.8 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.4 (2H, d, J=10.6 Hz), 7.25 (2H, d, J=10.6 Hz), 5.7 (1H, s), 5.6 (1H, s), 4.0 (6H, s), 3.2 (2H, q, J=8.5 Hz), 1.7 (2H, m), 1.5 (1H, m), 1.0 (6H, d, J=7.0 Hz).

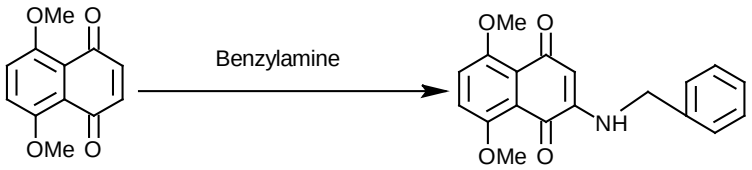
4k: 2-[2-(-Hydroxy-ethylamino)-ethylamino]-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc=1:4) to give the product. Yield: 38% (168.1 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.5 (1H, d, J=10 Hz), 7.2 (1H, d, J=10 Hz), 5.6 (1H, s), 4.2 (2H, t), 4.0 (6H, s), 3.8 (2H, t), 3.6 (2H, q), 2.0 (2H, q).

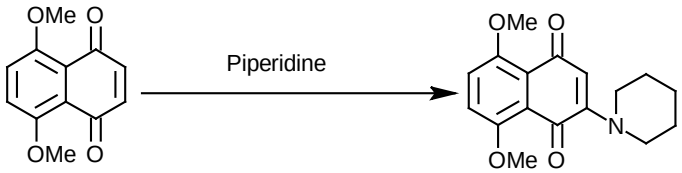
5k: 2-Benzylamino-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc=1:2) to give the product. Yield: 58.8% (262.6 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.5 (7H, m), 6.0 (1H, s), 5.6 (1H, s), 4.4 (2H, d, 6.8 Hz), 4.0 (6H, s).

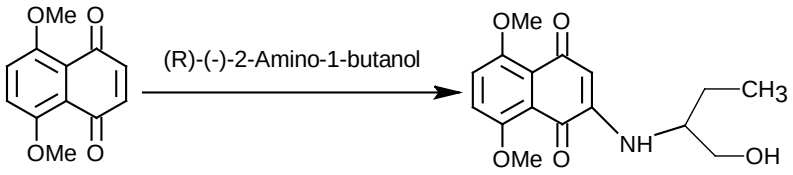
6k: 5,8-Dimethoxy-2-piperidin-1-yl-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc=1:2) to give the product. Yield: 33.3% (138.6 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.4 (1H, d, J=10.6 Hz), 7.3 (1H, d, J=10.6 Hz), 5.6 (1H, s), 4.0 (6H, s), 3.6 (2H, d, J=6 Hz), 2.6 (2H, t), 1.3 (6H, m).

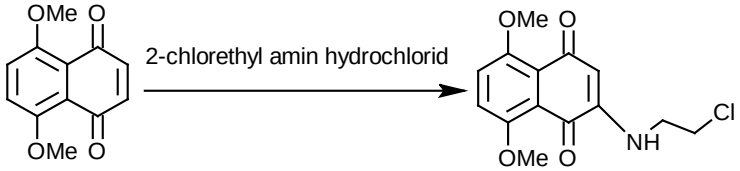
7k: 2-(1-Hydroxymethyl-propylamino)-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc=1:4) to give the product. Yield: 67.6% (285.1 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.4 (1H, d, J=10 Hz), 7.3 (1H, d, J=10 Hz), 5.7 (2H, s), 4.0 (6H, s), 3.7 (2H, d), 3.4 (1H, m), 2.5 (2H, m), 1.0 (3H, t, J=8.5 Hz).

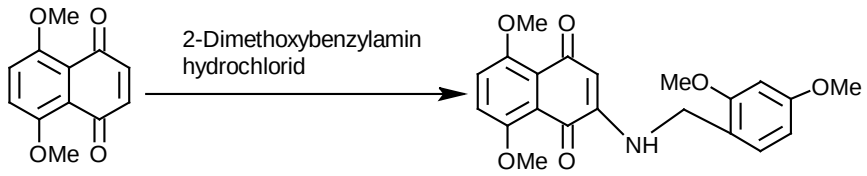
8k: 2-(2-Chloro-ethylamino)-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc=1:2) to give the product. Yield: 58.6% (239.2 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.5 (1H, d, J=10.7 Hz), 7.4 (1H, d, J=10.7 Hz), 6.0 (1H, t), 5.8 (1H, s), 4.5 (2H, q, J=9 Hz), 4.0 (6H, s), 3.5 (2H, t, J=7.0 Hz).

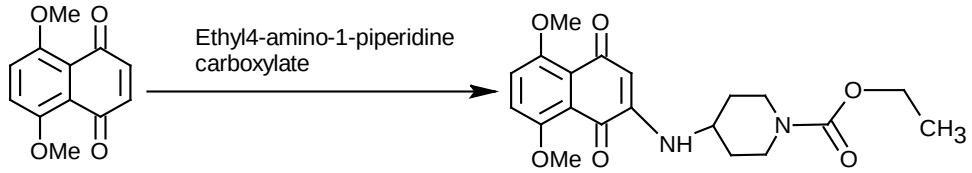
9k: 2-(2,4-Dimethoxy-benzylamino)-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc=1:2) to give the product. Yield: 41.8% (206.2 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.8 (1H, d, J=10 Hz), 7.6 (1H, s), 7.5 (1H, d, J=10 Hz), 6.5 (2h, d, J=8 Hz), 5.8 (1H, s), 4.5 (2H, d, J=7.0 Hz), 3.8 (6H, s), 3.6 (6H, s).

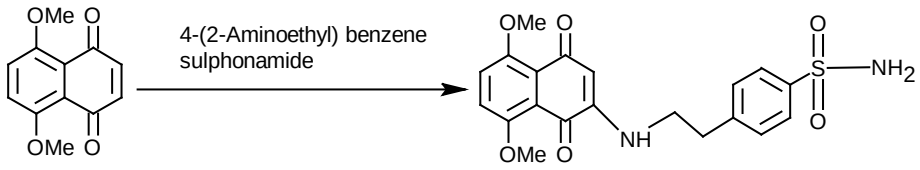
10k:4-(5,8-Dimethoxy-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-piperidine-1-carboxylic acid ethyl ester



The crude product was purified by column chromatography (hexanes:EtOAc=1:2) to give the product. Yield: 54.6% (238.4 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.5 (1H, d, J=10.3 Hz), 7.3 (1H, d, J=10.3 Hz), 5.6 (1H, s), 4.3 (2H, q, J=8 Hz), 2.1 (2H, t, J=8.7 Hz), 1.5 (2H, t, J=8 Hz), 1.3 (3H, t, J=7.3 Hz).

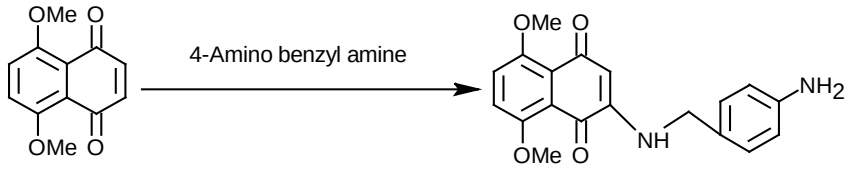
11k:4-[2-(5,8-Dimethoxy-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-ethyl]-benzensulfonamide



The crude product was purified by column chromatography (hexanes:EtOAc=1:2) to give the product. Yield: 90.7% (521.7 mg).

^{1}H-NMR (300Hz, DMSO) δ 7.8 (2H, d, J=9.3 Hz), 7.5 (4H, dd, J=10 Hz), 7.3 (2H, s), 7.0 (1H, t), 5.5 (1H, s), 3.8 (6H, s), 3.0 (2H, t, J=8.5 Hz), 2.5 (2H, t, J=8 Hz).

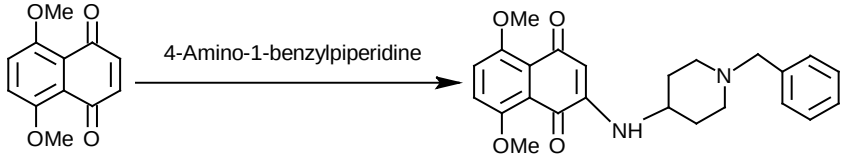
12k: 2-(4-Amino-benzylamino)-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc=1:2) to give the product. Yield: 81.9% (382.8 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.4 (1H, d, J=10.3 Hz), 7.3 (1H, d, J=10.3 Hz), 7.1 (2H, d, J=9.3 Hz), 6.7 (2H, d, J=9.3 Hz), 5.6 (1H, s), 4.2 (2H, d, J=9.7 Hz), 4.0 (6H, s), 3.7 (1H, s), 2.2 (2H, s).

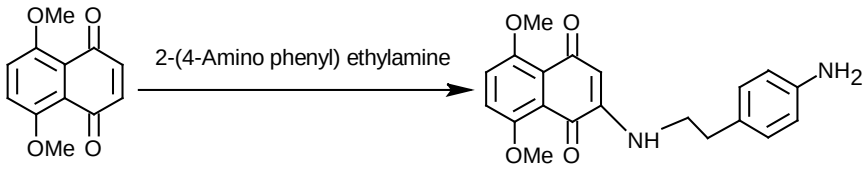
13k:2-(1-Benzyl-piperidin-4-ylamino)-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc=1:4) to give the product. Yield: 64.14% (359.8 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.5 (5H, m), 7.3 (2H, d, J=10.3 Hz), 5.65 (1H, d), 5.6 (1H, s), 4.0 (6H, s), 3.5 (2H, s), 3.4 (1H, m, J=8.7 Hz), 2.7 (2H, d, J=9 Hz), 2.3 (2H, t, J=9 Hz), 2.0 (2H, d, J=9 Hz) .

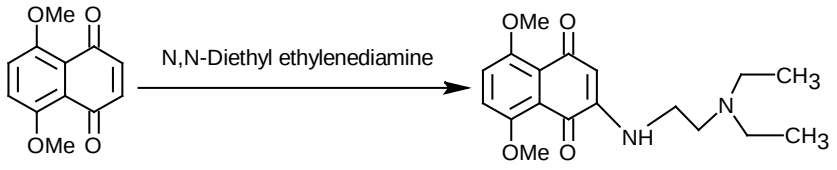
14k: 2-[2-(4-Amino-phenyl)-ethylamino]-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc=1:2) to give the product. Yield: 46.2% (225.1 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.5 (1H, d, J=10.35 Hz), 7.3 (1H, d, J=10.35 Hz), 7.0 (2H, d, J=10.3 Hz), 6.7 (2H, d, J= 10.3 Hz), 5.7 (1H, t), 5.6 (1H, s), 4.0 (6H, s), 3.5 (2H, q, J=8 Hz), 2.8 (2H, t, J=7.7 Hz).

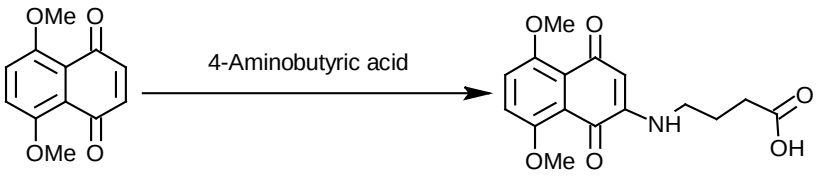
15k: 2-(2-Diethylamino-ethylamino)-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc=1:4) to give the product. Yield: 63.9% (284.5 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.9 (1H, d, J=10 Hz), 7.1 (1H, d, J=10 Hz), 6.6 (1H, s), 5.3 (1H, s), 4.0 (6H, s), 3.2 (4H, q, J=7 Hz), 2.6 (4H, t, J=6.8 Hz), 2.5 (6H, t, J=8 Hz).

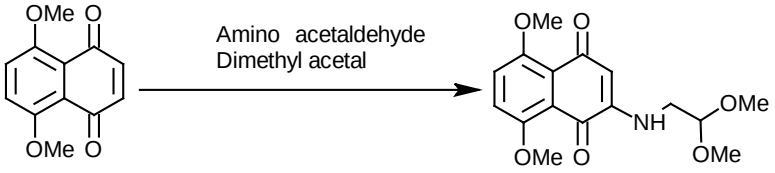
16k: 4-(5,8-Dimethoxy-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-bytyric acid



The crude product was purified by column chromatography (hexanes:EtOAc: MeOH=1:4:1) to give the product. Yield: 65.6% (289.1 mg).

^{1}H-NMR (300Hz, DMSO) δ 7.5 (1H, d, J=10.5 Hz), 7.4 (1H, d, J=10.5 Hz), 7.0 (1H, t), 5.5 (1H, s), 4.0 (6H, s), 3.1 (2H, q, J=7.7 Hz), 2.4 (2H, t, J=8 Hz), 1.7 (2H, q, J=7.7 Hz).

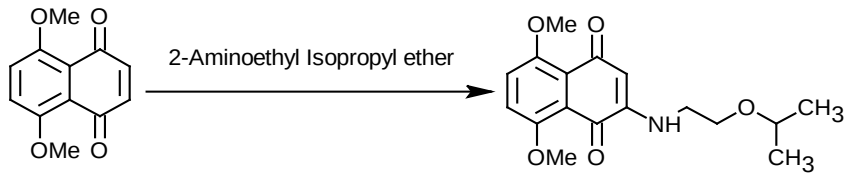
17k: 2-(2,2-Dimethoxy-ethylamino)-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc=1:2) to give the product. Yield: 65.2% (370.7 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.4 (1H, d, J=10.3 Hz), 7.3 (1H, d, J=10.3 Hz). 5.9 (1H, s), 5.6 (1H, s), 4.6 (1H, t, J=9.3 Hz), 4.0 (6H, s), 3.5 (6H, s), 3.3 (2H, t, J=6.8 Hz).

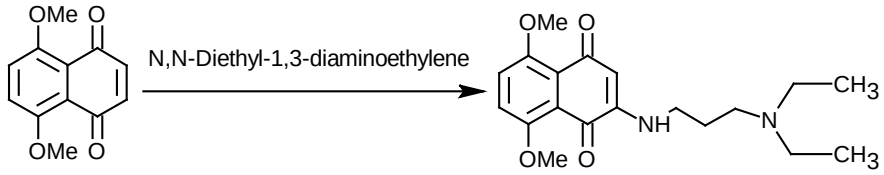
18k: 2-(2-Isopropoxy-ethylamino)-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc=1:2) to give the product. Yield: 37% (244.8 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.5 (1H, d, J=10.3 Hz), 7.3 (1H, d, J=10.3 Hz), 6.1 (1H, m), 5.6 (1H, s), 4.0 (6H, s), 3.6 (2H, t, J=7 Hz), 3.5 (1H, m), 3.3 (2H, q, J=6.8 Hz), 1.6 (6H, d, J=7 Hz).

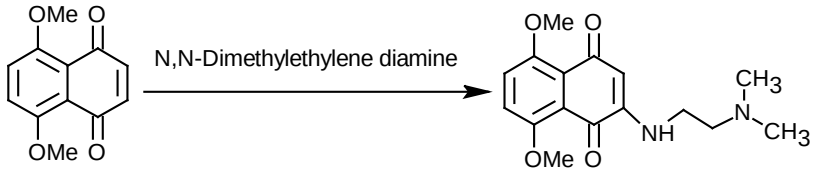
19k: 2-(3-Diethylamino-propylamino)-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc:MeOH=1:3:1) to give the product. Yield: 58.58% (420 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.4 (1H, d, J=10.6 Hz), 7.3 (1H, d, J=10.3 Hz), 6.8 (1H, s), 5.6 (1H, s), 4.0 (6H, s), 3.3 (2H, q, J=6.8 Hz), 2.5 (6H, m), 1.8(2H, q, J=7.3 Hz), 1.0 (6H, t, J=7.7 Hz).

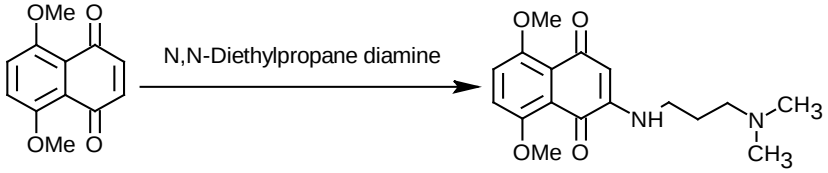
20k: 2-(2-Dimethylamino-ethylamino)-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc:MeOH=1:3:1) to give the product. Yield: 56.76% (357.6 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.4 (1H, d, J=10.6 Hz), 7.2 (1H, d, J=10.6 Hz), 6.3 (1H, s), 5.6 (1H, s), 4.0 (6H, s), 3.2 (2H, q, J=9.3 Hz), 2.5 (2H, t, J=6.8 Hz), 2.3 (6H, s).

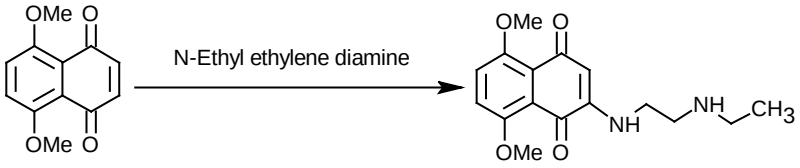
21k: 2-(3-Dimethoxylamino-propylamino)-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc:MeOH=1:3:1) to give the product. Yield: 64.6% (284.1 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.4 (1H, d, J=10 Hz), 7.3 (1H, d, J=10 Hz), 6.6 (1H, s), 5.6 (1H, s), 4.0 (6H, s), 3.2 (2H, q, J=8 Hz), 2.45 (2H, q, J=7 Hz), 2.3 (6H, s), 1.7 (2H, q, J=7 Hz).

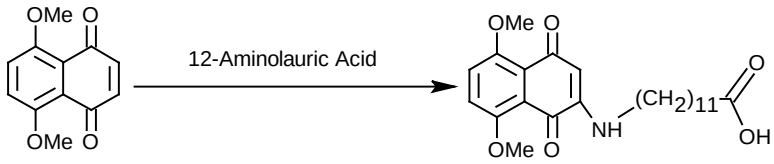
22k: 5,8-Dimethoxy-2-(2-methylamino-ethylamino)-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc:MeOH=1:4:1) to give the product. Yield: 54.2% (228 mg).

^{1}H-NMR (300Hz, DMSO) δ 7.5 (1H, d, J=10 Hz), 7.4 (1H, d, J=10 Hz), 7.0 (1H, t, J=6.8 Hz), 5.5 (1H, s), 3.8 (6H, s), 3.6 (2H, q, J=7.3 Hz), 2.3 (4H, t, J=6.0 Hz), 1.7 (3H, t, J=7.7 Hz).

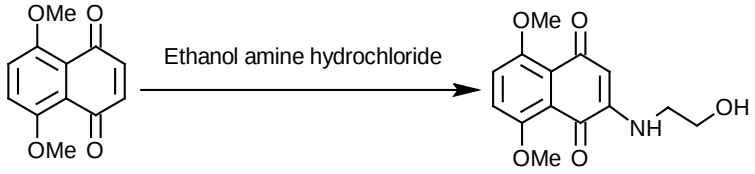
23k:12-(5,8-Dimethoxy-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-dodecanoic acid



The crude product was recrystallized by MeOH to give the product. Yield: 45.2% (269 mg).

^{1}H-NMR (300Hz, DMSO) δ 7.5 (1H, d, J=10.7 Hz), 7.4 (1H, d, J=10.7 Hz), 7.0 (1H, t, J=6.8 Hz), 5.4 (1H, s), 3.8 (6H, s), 3.0 (2H, q, J=7.3 Hz), 2.2 (2H, q, J=8.3 Hz), 1.3 (18H, m).

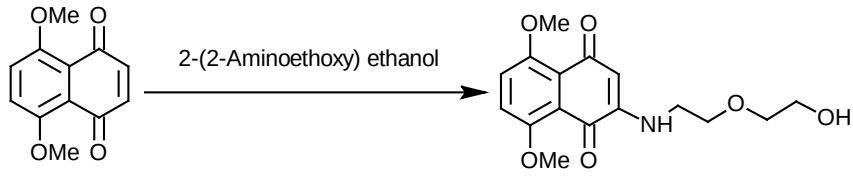
24k: 2-(2-Hydroxy-ethylamino)-5,8-dimethoxy-[1,4]naphthoquinones



The crude product was purified by column chromatography (hexanes:EtOAc=1:4) to give the product. Yield: 64.2% (362.8 mg).

^{1}H-NMR (300Hz, DMSO) δ 7.5 (1H, d, J=11 Hz), 7.4 (1H, d, J=11 Hz), 6.0 (1H, t, J=6.8 Hz), 5.5 (1H, s), 3.8 (6H, s), 3.1 (2H, t, J=9.3 Hz).

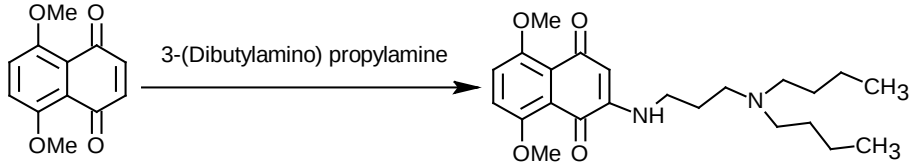
25k: 2-[2-(2-Hydroxy-ethoxy)-ethylamino]-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOAc:MeOH=1:4:1) to give the product. Yield: 67.2% (297.8 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.45 (1H, d, J=10.3 Hz), 7.4 (1H, d, J=10.3 Hz), 6.0 (1H, t, J=7.7 Hz), 5.6 (1H, s), 4.0 (6H, s), 3.7 (2H, t, J=8.3 Hz), 3.5 (2H, t, J=8.3 Hz), 2.8 (2H, q, J=6.3 Hz).

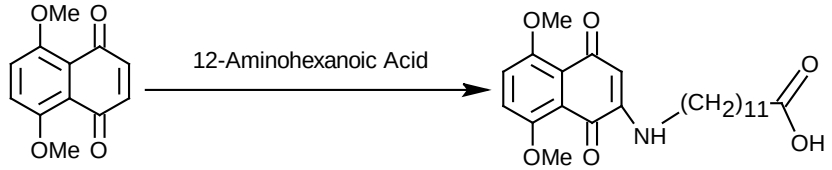
26k: 2-(3-Dibutylamino-propylamino)-5,8-dimethoxy-[1,4]naphthoquinone



The crude product was purified by column chromatography (hexanes:EtOA=1:2) to give the product. Yield: 66.2% (367.8 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.4 (1H, d, J=10.7 Hz), 7.3 (1H, d, J=10.7 Hz), 6.6 (1H, s), 5.6 (1H, s), 3.9 (6H, s), 3.3 (2H, q, J=6.3 Hz), 2.5 (2H, t, J=7.0 Hz), 2.4 (4H, t, J=7.3 Hz), 1.5 (2H, q, J=8.0 Hz), 1.4 (4H, t, J=6Hz), 1.3 (4H, t, J=8.0 Hz), 1.0 (6H, t, J=8.0 Hz).

27k: 6-(5,8-Dimethoxy-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-hexanoic acid

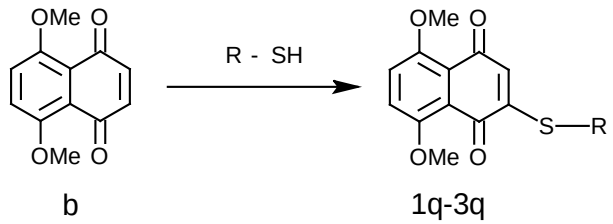


The crude product was recrystallized by MeOH to give the product. Yield: 44.2% (212 mg).

^{1}H-NMR (300Hz, DMSO) δ 7.5 (1H, d, J=10 Hz), 7.4 (1H, d, J=10 Hz), 7.0 (1H, t, J=7.0 Hz), 5.4 (1H, s), 4.0 (6H, s), 3.0 (2H, t, J=7.7 Hz), 2.2 (2H, t, J=7.7 Hz), 1.5 (4H, q, J=8.7 Hz), 1.3 (2H, q, J=7.3 Hz).

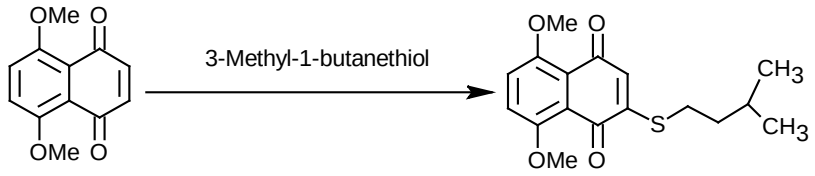
5.3.2 General produce for the synthesis of compounds (1q-3q)

To a solution of 5,8-Dimethoxynaphthalene-1,4-dione (f) (301 mg, 1.38 mmol) in MeOH (30 ml) were added corresponding alkyl thiols (1.65 mmol). The mixture was stirred at room temperature for 4h and to the solution was added dropwise a solution of sodium dichlromate (0.23 mmol) and sulfuric acid (0.76 mmol) in water. The resulting mixture was stirred for a few minute and the acidic solution was then extracted with dichloromethane (50 ml x3). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and then concentrated under reduced pressure. The residue was chromatography (hexane:EtOAc) to give title compounds **1q**-**3q**.



b 1q-3q

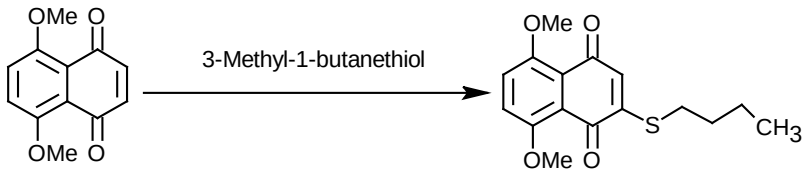
1q: 5,8-Dimethoxy-2-(3-methyl-butylsulfanyl)-[1,4]naphthoquinone



To solution of 5,8-Dimethoxynaphthalene-1,4-dione (301mg, 1.38mmol) in MeOH (30mL) were added 3-Methyl-1-butanethiol (256.8µl, 1.65 mmol). The mixture was stirred at room temperature for 4h and to the solution was added dropwise a solution of sodium dichlromate (0.23 mmol) and sulphuric acid (0.76 mmol) in water. The resulting mixture was stirred for a few minutes and the acidic solution was then extracted with dichlromethane (50mL ×3). The combined organic layers were washed with brine, dried over anhydrous sodium sulphate, filtered, and then concentrated under reduced pressure. The residue was purified by column chromatography (hexanes:EtOAc=5:1) to give the product. Yield: 71.5% (404.9 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.5 (2H, d, J=10.3 Hz), 4.0 (6H, s), 2.6 (2H, t, J=8 Hz), 1.8 (1H, m), 1.5 (2H, m, J=7.3 Hz), 1.0 (6H, d, J=7.4 Hz).

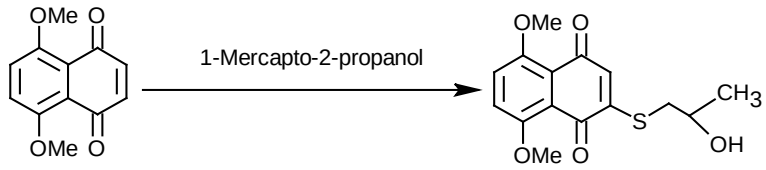
2q: 2-Butylsulfanyl-5,8-dimethoxy-[1,4]naphthoquinone



The residue was purified by column chromatography (hexanes:EtOAc=5:1) to give the product. Yield: 67.9% (287.2 mg).

^{1}H-NMR (300Hz, $CDCl_3$) δ 7.4 (2H, d, J=10.6 Hz), 4.0 (6H, s), 2.7 (2H, t, J=7.3 Hz), 1.7 (2H, m), 1.5 (2H, m), 1.0 (3H, t, J=8 Hz).

3q: 2-(2-Hydroxy-propylsulfanyl)-5,8-dimethoxy-[1,4]naphthoquinone



The residue was purified by column chromatography (hexanes:EtOAc=1:4) to give the product. Yield: 75% (319.2 mg).

^{1}H-NMR (300Hz, DMSO) δ 7.5 (2H, d, J=9.3 Hz), 6.5 (1H, s), 5.1 (2H, d, J=6.3), 3.85 (6H, s), 2.8 (1H).

III. RESULTS AND DISCUSSION

Scheme 1 shows the general synthesis routes for the starting materials. 2-formyltetramethoxynaphthalene **(a)** was prepared from 1,5-dihydroxynaphthalene **(1)** through 4-step reactions of methylation (63%), bromination (69%), methoxylation (62.5%) and formylation (96%). 2-formyltetramethoxynaphthalene was condensed with substituted anilines at pH 5 and the resulting imine compounds were reduced to amine compounds **(1d-5d)** using LAH in good yields. We could obtain 6-substituted naphthoquinone derivatives **(1c-6 ~ 5c-6)** with a yield of 63-80.8% by oxidation with chromium (VI) oxide and 2-substituted naphthoquinone derivatives **(1c-2 ~ 5c-2)** with a yield of 63-80.8% by oxidation with cerium (IV) ammonium nitrate (CAN) from corresponding 2-substituted-1,4,5,8-tetramethoxy naphthalenes **(1c-5c)** (scheme II).

The synthetic routes for 2-substituted amino-5,8-dimethoxy-1,4-naphthoquinone derivatives and 2-substituted thio-5,8-dimethoxy-1,4-naphthoquinone derivatives were summarized in Scheme I and Scheme III. 1,5-dihydroxynaphthalene (**1**), as a starting material, also was reacted with sodium hydroxide and dimethyl sulphate under nitrogen followed by bromination with N-bromosuccinimide (NBS) in room temperature for 3h to afford 1,5-dibromo-4,8-dimethoxy-naphthalene (**3**). After methoxylation with sodium methoxide and copper (I) iodide in N,N-dimethyl formamide/methanol solution, oxidative demethylation of the 1,4,5,8-tetramethoxy-naphthalene (**4**) with cerium (IV) ammonium nitrate (CAN) gave the key intermediate 5,8-dimethoxy-1,4-naphthoquinone (DMNQ, **b**). Direct 1,4-type addition of various alkylamines and arylamines to the quinone moiety of DMNQ **(b)** yielded the appropriated 2-amino-DMNQs, **1k-27k** with yields varying from 37 to 81.9%. Also, direct 1,4-type addition of various alkylthiols or arylthiols to the quinone moiety of DMNQ **(b)** yielded the appropriated 2-thio-DMNQs, **1q-3q.**

The different NQ derivatives were evaluated for their algicidal activity against harmful algae. The specificity and potency of all 40 NQ compounds (Table 1) against *Microcystic aeruginos, Anabaena flos-aquae, Stephanodiscus hantzschii, Cyclotella, Peridinium bipes* were determined. The synthesized compounds were tested under the assay conditions at various

micro molar concentrations, and the results and statistical significance were verified. Their inhibitory activity (IC_{50}) values are listed in Table 2. When algicidal activity of the NQ to *Microcystic aeruginos, Anabaena flos-aquae, Stephanodiscus hantzschii, Cyclotella, Peridinium bipes* were compared, some of NQ required high concentrations to inhibit growth, whereas a number of NQ showed moderate algicidal activity at the lowest concentrations against the green tides causing alga selected for this study.

As show in Table 2, the synthesized 2-substituted and 6-substituted DMNQs were measured against algae activity. It could be recognized that almost NQ compounds showed the potent activity to against the HABs. NQ compounds such as, **3c-6**, **4c-6**, **4c-2**, **5c-6**, **5c-2** exhibited IC_{50} values < 2µM for all HABs studied which makes them possible candidates for algicidal application. In contrast, compound **2c-2** exhibited relatively high IC_{50} values ranging from 4 - 40 µM, which suggest that the HABs are tolerant to these NQs. Among the compounds tested, compounds **1c-2**, **1c-6**, **2c-6**, **3c-2** exhibited IC_{50} of approximately 2 – 20 µM for all dianoflagellates. These compounds were potent for both HABs and none-harmful dianoflagellates indicating that these compounds are unsuitable as algicidal compounds (Table 2).

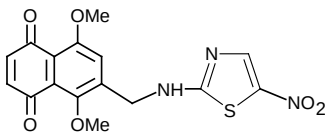
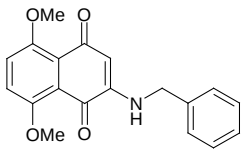
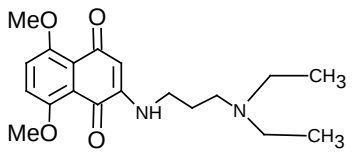
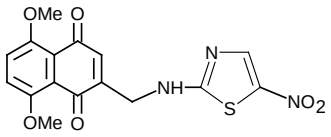
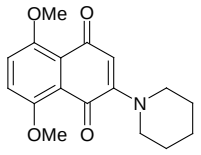
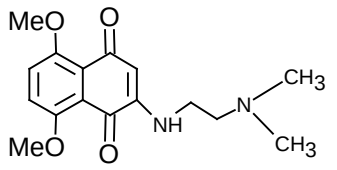
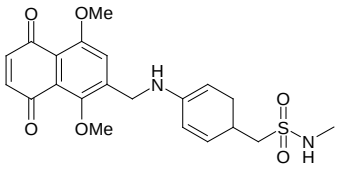
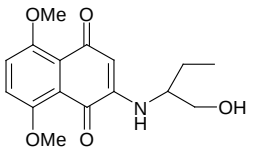
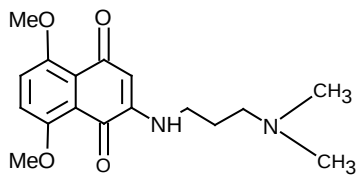
In comparison, the relationship between structure and activity, it was found that almost 6-substituted DMNQ exhibited higher algicidal activity against harmful algae than 2-substituted DMNQ (Table 2). However, interesting, it was observed that 2-substituted compound **5c-2** which has ethyl nitrobenzoyl acetataly groups at position, exhibited a better algicidal activity than 6-substituted compounds against harmful algae. The IC_{50} value of 2-substituted compound **5c-2** against algicidal activity also was found that better than others. This compound **5c-2** were extremely competent and selective against 5 categories harmful algae blooms including *Microcystic aeruginosa, Anabaena flos-aquae, Stephanodiscus hantzschii, Cyclotella* and *Peridinium bipes* with exhibited an IC_{50} ranging from 0.04 to 0.78µM, while non-harmful aglae *Aulacoseira granulata* and *Scenedesmus actus* showed an IC_{50} value > 40µM. So, this compound is the most potential candidate for control of HABs. In earlier works, we observed that the algicidal activity was dependent upon the location of the substituent groups. In the case of NQ derivatives with substituent functional group at C6 and C2 position, we had shown that 6-substituted derivatives were more effective than the 2-substituted derivatives. This result was in accord with the works reported by other researchers and it was

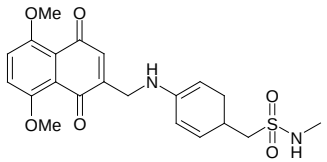
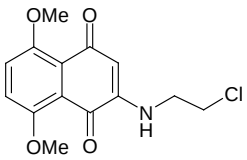
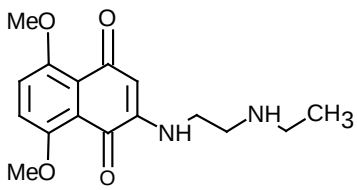
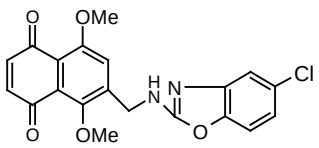
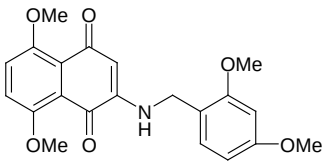
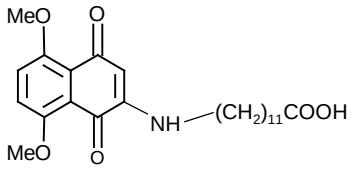
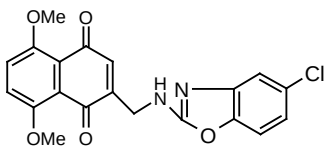
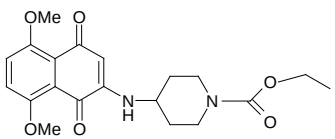
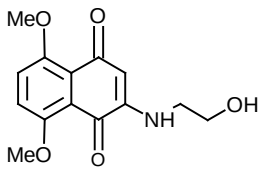
said that the C2 or C3 of 6-substituted compounds would be better Michael acceptors than the C3 of 2-substituted compounds and attacked more easily by nucleophiles such as amine or thiol functional groups in the cell [40, 41]. As evidenced in earlier reports [39, 40] steric hindrance of C-2 substituent of the naphthoquinone derivatives may explain the lower algicidal activity of 2-substituted derivatives. Also, electron density in the quinoid ring may be important. Previously, it had been reported that an electron withdrawing group such as acetoxy or oxo group at C1 in side chain of naphthoquinone analogues enhanced activity [40]. Compounds **5c-2**, which possess a higher electrophilicity of the C3 position, was found to be good algicidal activity against harmful algae than others. The relatively high algicidal activity of 2-substituted compound **5c-2**, despite steric hindrance could be explained by the assumption that steric effect of side chain at C2 position could be compensated by the electrophilicity of the C3 position. It was also observed that 6-substituted derivatives showed better algicidal activity than 2-substituted derivatives. The main cause of lowered activities of 2-substituted derivatives was conducted by the steric hindrance of the quinonoid moiety. This result may suggest that these compounds with oxo group such as **5c-2** and **5c-6** are more suitable for the inhibition of algicidal activity than others. Therefore, **5c-2** *(Ethyl2-(4-nitrobenzol)-3-(5,8-dimethoxy-1,4-dioxo-1,4-dihydro-2-naphthalenyl)-2-propenoate)* may prove useful for the design of new potent HABs inhibitors.

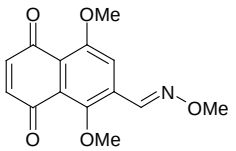
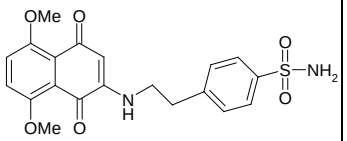
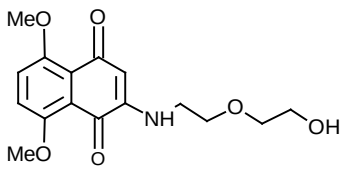
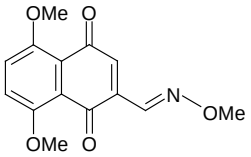
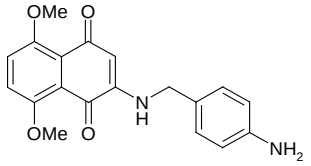
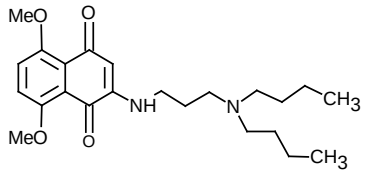
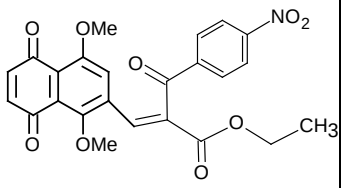
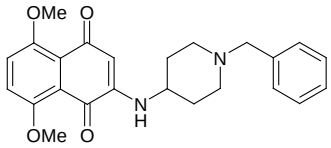
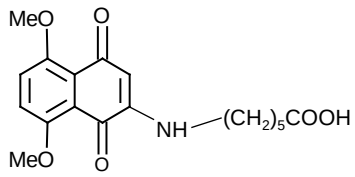
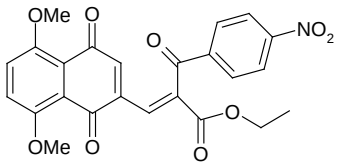
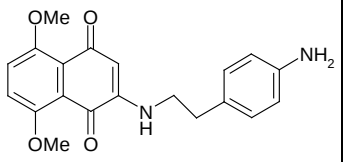
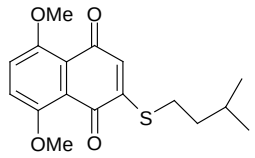
As shown in Table 2, the synthesized 2-amino-DMNQ analogues **(1k-27k)** and 2-thio-DMNQ anologues **(1q-3q)** were evaluated for their inhibitor in harmful algae. It was notable that the inhibitory effect of the 2-substituted DMNQs against harmful algal blooms was dependent on having the oxygen atoms or sulphur atoms or nitrogen atoms present in alkyl groups. Of the compounds were conducted, compounds **1k**, **15k**, **19k**, **20k**, **21k**, **26k**, **2q**, and **3q** were most potent against *Microcystic aeruginos* exhibiting IC_{50} values < 1μM. In contrast, some compounds such as **4k**, **6k**, **9k**, **10k**, and **14k** showed high IC_{50} values ranging from 10 to 40μM which makes them unsuitable candidates for against HABs. In particular, compounds **2k**, **3k**, **13k**, **18k**, and **25k** were extremely competent and selective against harmful algae *Anabaena flos-aquae* with exhibited an IC_{50} ranging from 0.6 to 1μM, while *Microcystic aeruginosa* and *Cyclotella* showed an IC_{50} value ranging from 3 to 40μM. Therefore, these compounds may have structural compentence to *Anabaena flos-aquae* but the mechanism of this selectivity need to be examined further. In comparison, the relationship between structure

and activity, we found that the diverse algicidal effects caused by incorporation of oxygen, sulphur, or nitrogen atoms with naphthoquinone ring. A number of 2-substituted DMNQs such as **15k**, **19k**, **20k**, **21k**, **26k**, **2q**, **3q** having nitrogen atoms and sulphur atoms present in them have been shown with the most potency against harmful algicides *(Microcystic aeruginos , Anabaena flos-aquae, Stephanodiscus hantzschii, Cyclotella, Peridinium bipes)* while non-harmful algae (*Aulacoseira granulate Scenedesmus actus)* were comparatively less affected by these compounds. These results indicate that these groups are the most suitable candidate for the control HABs. Next, we synthesized and assayed the 2-substituted DMNQs with oxygen atoms and aromatic ring as substituent instead of aliphatic linear chain. However, compound **9k** did not exhibit inhibition of algicidal activity. In addition, in order to improve solubility of DMNQ derivatives in water, we introduced hydroxyl group (**4k**, **7k**, **25k**), carboxylic group (**16k**, **23k**, **27k**) and amine group (**11k**, **12k**, **14k**) instead of methyl group in the terminal with the less inhibitory effect on algicidal activity. The results indicate that hydrophobic groups are more suitable than the hydrophilic groups in the terminal of DMNQ analogues. On the basic of this observation, compound **3q** (*2-(2-Hydroxy-propylsulfanyl)-5,8-dimethoxy-[1,4]naphthoquinone*) showed the most potent compound with the less IC_{50} values in this series of 2-substituted DMNQs. This compound **3q** were extremely competent and selective against harmful algae blooms including *Microcystic aeruginosa, Anabaena flos-aquae, Stephanodiscus hantzschii,* and *Cyclotella* with exhibited an IC_{50} ranging from 0.13 to 2.2µM, while non-harmful aglae *Aulacoseira granulata* and *Scenedesmus actus* showed an IC_{50} value > 50µM. So, this compound is the most potential candidate for control of HABs and also safe to non-harmful algae. These results may suggest that hydrophobic groups such as nitrogen atoms and sulphur atoms are more suitable for the inhibition of algicidal activity than hydrophilic group like –OH (**4k**, **7k**, **25k**) or –COOH (**16k**, **23k**, **27k**) and $–NH_2$ group (**11k**, **12k**, **14k**). The Table 2 gives a summary of the inhibitory potency of all these compounds.

Table 1. The various synthetic naphthoquinones

1c-6		**5k**		**19k**	
1c-2		**6k**		**20k**	
2c-6		**7k**		**21k**	

2c-2		**8k**		**22k**	
3c-6		**9k**		**23k**	
3c-2		**10k**		**24k**	

4c-6		**11k**		**25k**	
4c-2		**12k**		**26k**	
5c-6		**13k**		**27k**	
5c-2		**14k**		**1q**	

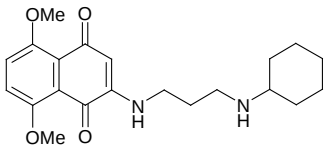
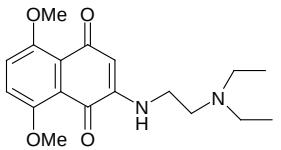
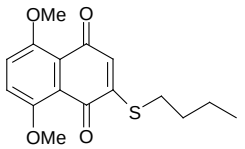
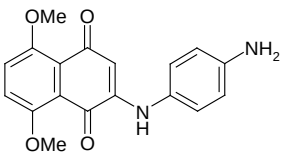
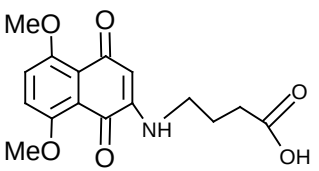
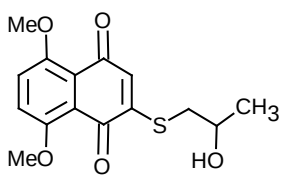
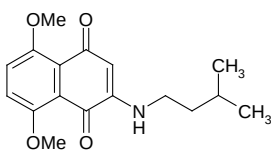
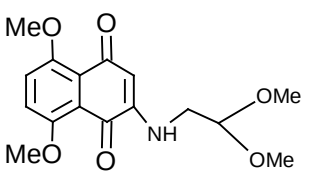
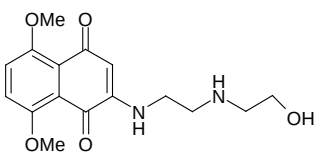
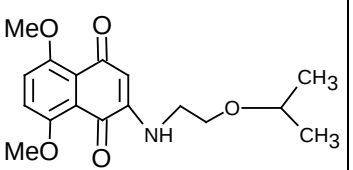
1k		**15k**		**2q**	
2k		**16k**		**3q**	
3k		**17k**			
4k		**18k**			

Table 2. Algicidal effects of the various synthetic naphthoquinones

S.No	IC_{50} (µM)						
	Harmful algal species					Non-harmful algal species	
	Microcystic aeruginosa	Anabaena flos-aquae	Stephanodiscus hantzschii	Cyclotella	Peridinium bipes	Aulacoseira granulata	Scenedesmus actus
1c-6	9.4	0.29	3.5	7.0	>50	48.4	>50
1c-2	7.5	0.9	1.9	7.3	>50	0.9	>50
2c-6	1.5	1.4	3.7	17.6	13.5	>50	>50
2c-2	38.8	3.7	3.8	15.2	9.6	>50	>50
3c-6	0.8	0.61	0.42	1.7	>50	46.9	>50
3c-2	7.0	0.17	0.5	0.92	>50	47.0	>50

4c-6	0.55	0.38	0.04	0.34	0.75	>50	>50
4c-2	0.56	0.67	0.21	0.55	0.95	40.2	>50
5c-6	0.66	0.47	0.06	0.42	0.85	44.3	>50
5c-2	0.32	0.09	0.04	0.7	0.78	40.5	>50
1k	0.53	0.8	1.7	10.5	2.5	>50	>50
2k	3.1	0.7	1.3	7.7	0.91	>50	>50
3k	3.1	1.3	1.9	7.3	0.86	>50	>50
4k	37.7	4.1	9.9	39.6	18.3	>50	>50
5k	7.9	1.4	3.4	7.3	3.6	>50	>50
6k	15.3	3.2	4.6	17.4	2.5	>50	>50
7k	9.2	0.9	9.2	38.6	9.5	48.6	>50
8k	3.5	1.5	1.3	7.1	4.5	>50	>50
9k	44.8	1.9	9.9	48.9	9.8	>50	>50
10k	9.0	0.9	3.6	5.5	8.5	>50	>50
11k	8.9	1.4	1.7	3.7	2.4	>50	>50
12k	7.7	1.5	1.4	3.7	10.8	47.3	>50
13k	7.4	0.8	3.6	7.2	1.2	44.6	>50
14k	19.2	2.7	4.0	5.5	37.4	>50	>50
15k	0.16	0.37	1.7	6.2	13.1	>50	>50

16k	4.2	4.1	18.4	>50	6.7	>50	>50
17k	2.2	0.61	0.9	11.1	5.3	>50	>50
18k	3.0	0.60	1.6	8.6	3.8	48.5	>50
19k	0.66	0.37	4.3	24.0	11.2	>50	>50
20k	0.18	0.17	4.2	7.4	2.4	>50	>50
21k	0.22	1.3	2.3	9.1	1.7	>50	>50
22k	3.8	4.2	7.5	>50	7.3	>50	>50
23k	4.2	7.6	3.6	21.0	1.7	>50	>50
24k	1.4	0.85	1.2	7.4	1.6	45.3	>50
25k	3.1	0.75	13.0	>50	48.3	>50	>50
26k	0.71	2.1	5.0	8.3	4.4	>50	>50
27k	7.8	10.5	3.3	46	24.0	>50	>50
1q	7.0	1.6	0.4	3.5	12.0	>50	>50
2q	0.76	3.8	0.6	0.78	40.2	>50	>50
3q	0.13	0.31	1.2	2.2	8.8	>50	>50

IV. CONCLUSION

In summary, conducted naphthoquinone derivatives as potential algicidal agents. The synthesis of 40 NQ compounds provide a strong evidence that several NQ derivatives can have effective algicidal activity against HABs, but are safe to non-harmful algae. A few of the NQ exhibited remarkable algicidal activity against *Microcystic aeruginos, Anabaena flos-aquae, Stephanodiscus hantzschii, Cyclotella, Peridinium bipes* with low IC_{50} value (0.04 to 2µM). The most potent inhibitors of this series of compounds against *Microcystic aeruginos, Anabaena flos-aquae, Stephanodiscus hantzschii, Cyclotella, Peridinium bipes* are compounds **4c-6**, **4c-2**, **5c-6**, and **5c-2** with IC_{50} lower than 1µM, while non-harmful aglae *Aulacoseira granulata* and *Scenedesmus actus* showed an IC_{50} value > 40µM. In contrast, some compounds such as **4k**, **6k**, **9k**, **10k**, and **14k** showed high IC_{50} values ranging from 10 to 40µM which makes them unsuitable candidates for against HABs. Insights regarding the use of NQ compounds may not be limited or restrict by the growth of the other organisms due to their selectivity. A precise insights into the inhibitory action against the green tide algal remains to be determined. In addition, there are many aspects of potent or competent algal inhibitors in the treatment of harmful algal blooms that need to be clarified including safety, lifetime, water solubility, and stability.

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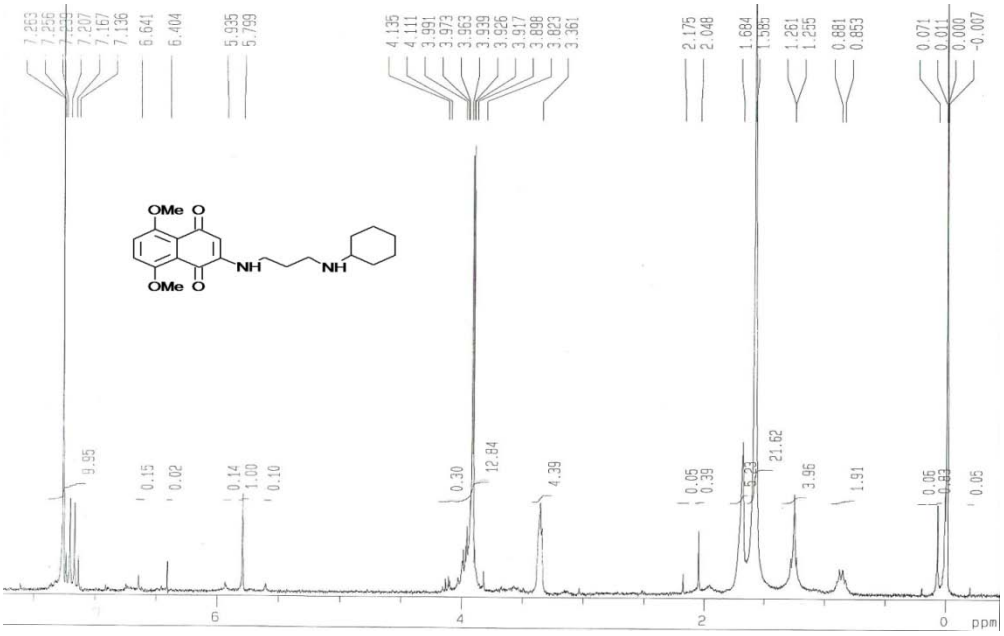
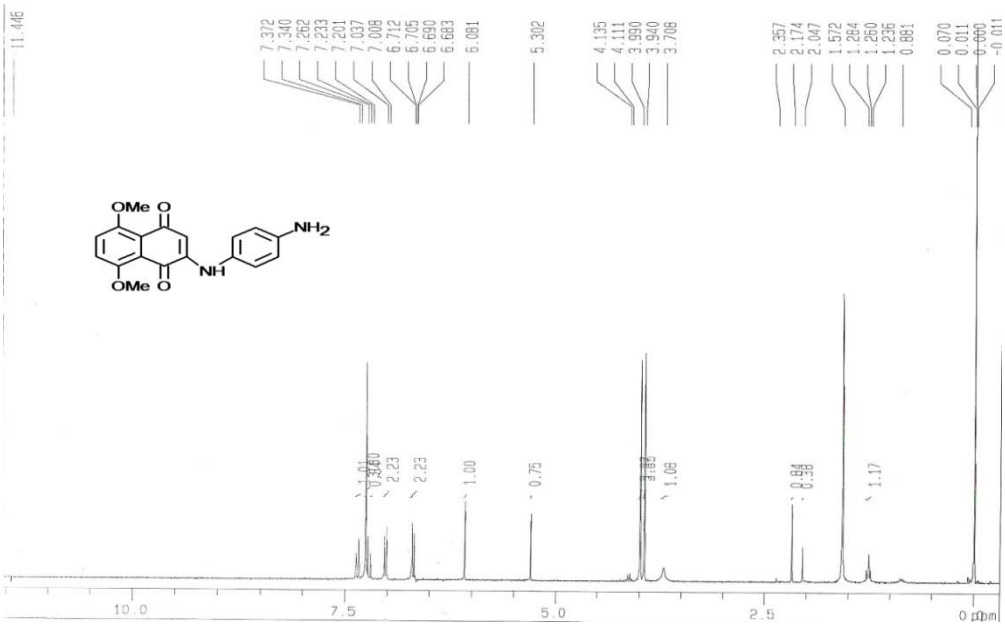
^{1}H NMR Spectra



1k: 2-(3-Cyclohexylamino-propylamino)-5,8-dimethoxy-[1,4]naphthoquinone



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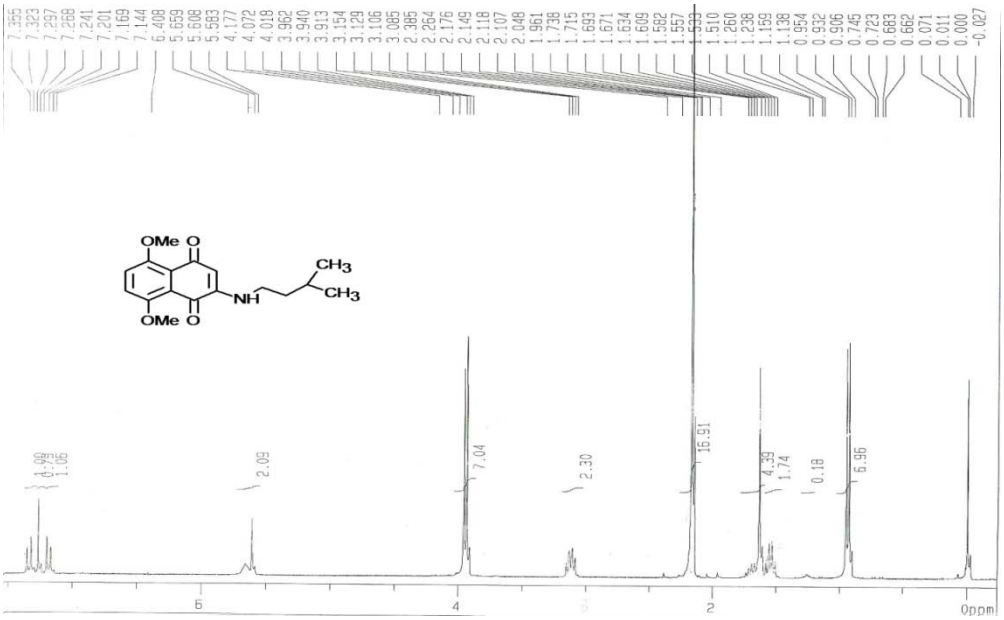
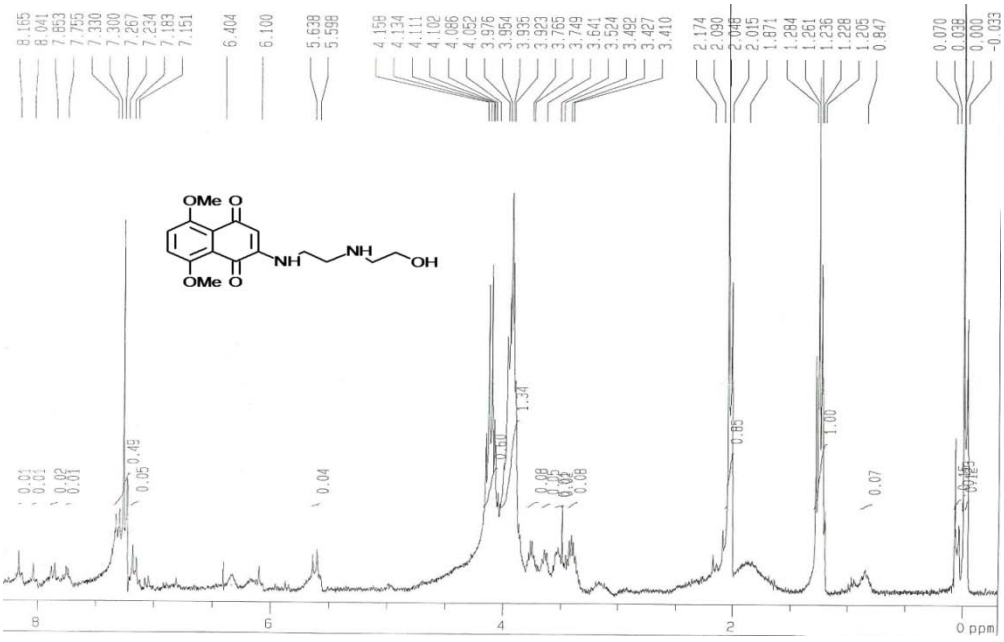
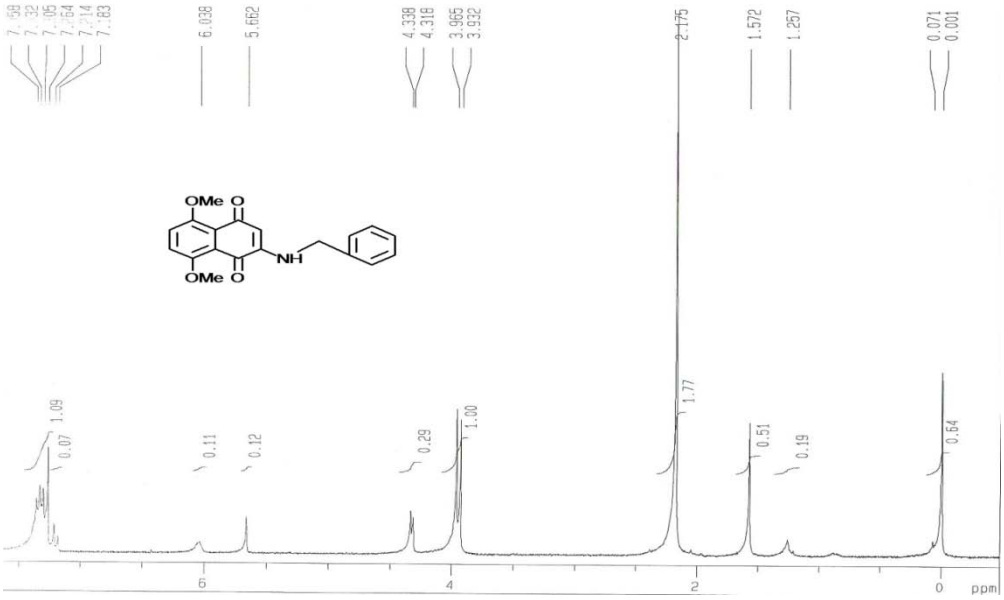


3k: 5,8-Dimethoxy-2-(3-methyl-butylamino)-[1,4]naphthoquinone



4k: 2-[2-(2-Hydroxy-ethylamino)-ethylamino]-5,8-dimethoxy-[1,4]naphthoquinone



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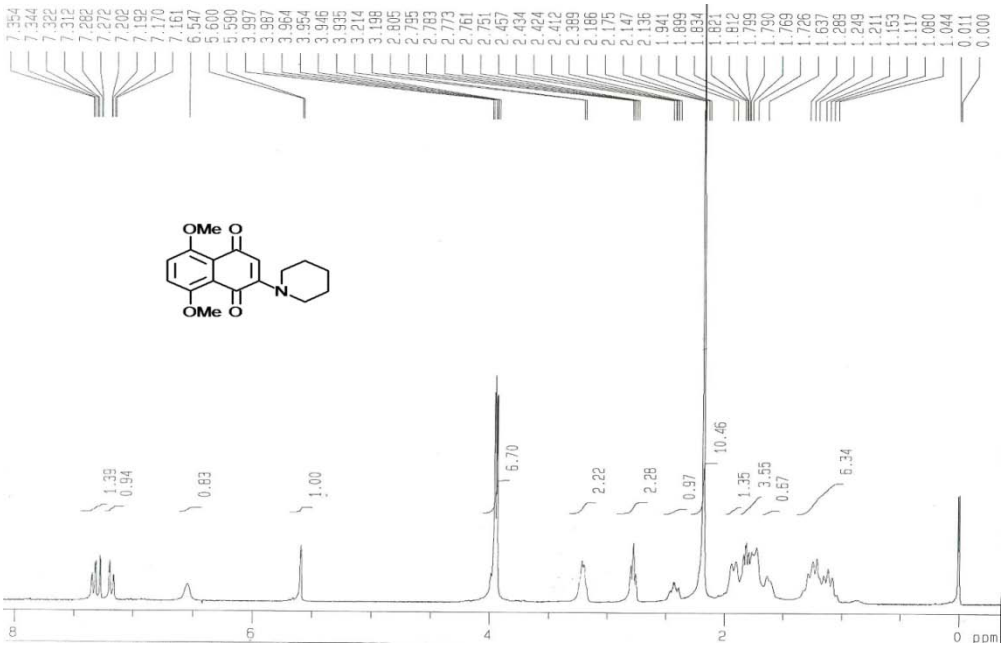
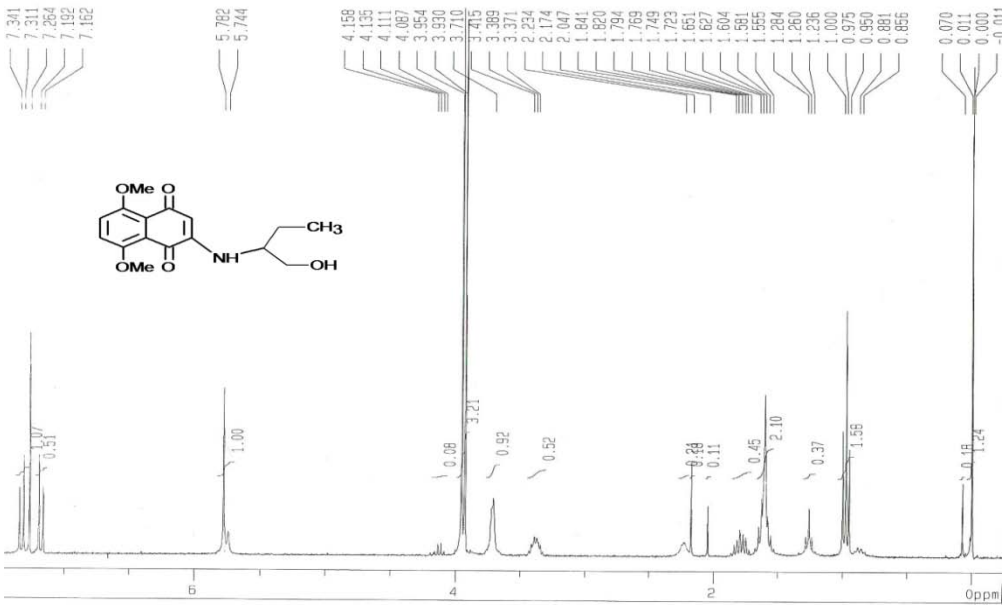
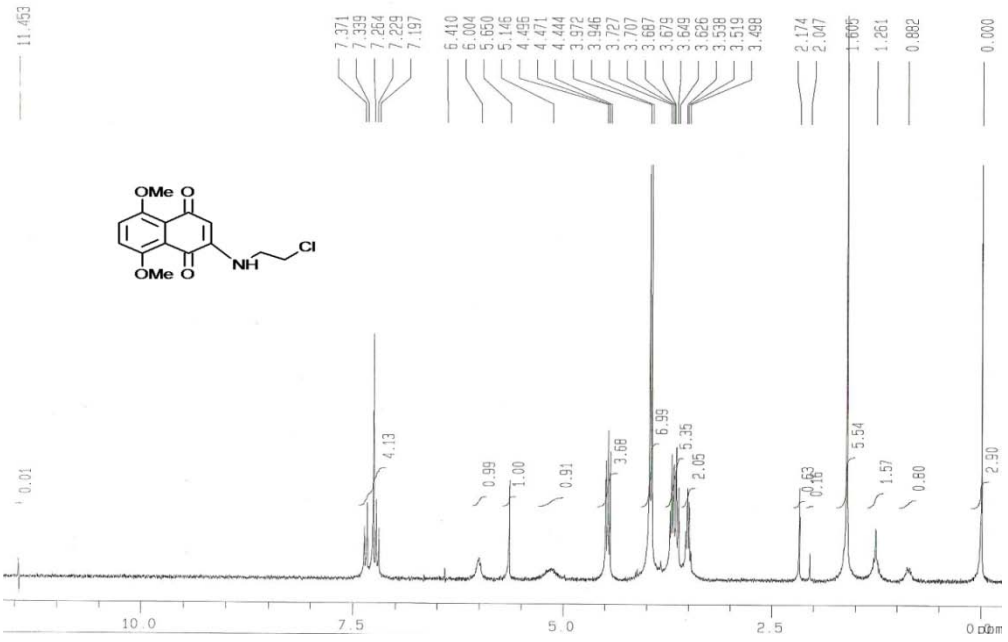


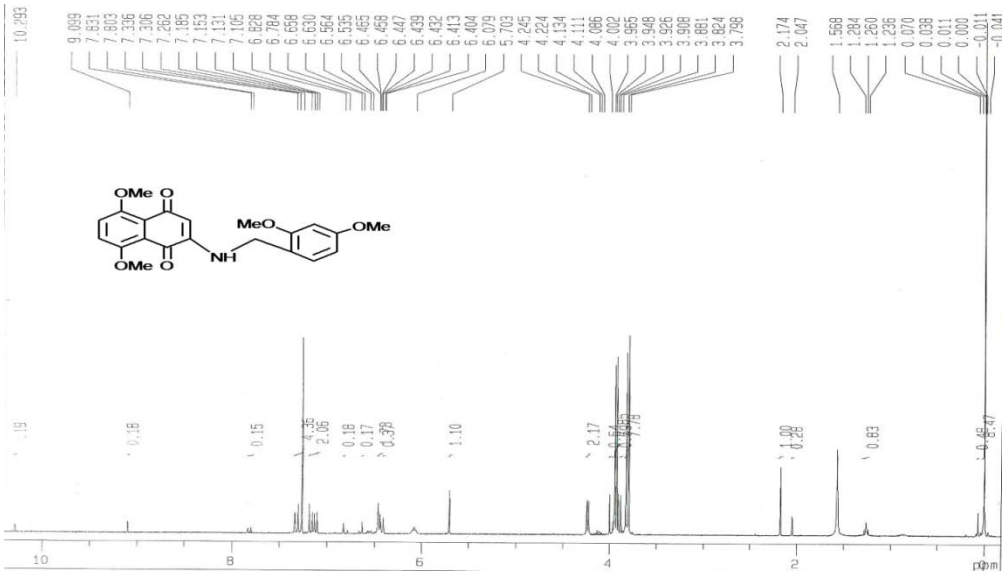
6k: 5,8-Dimethoxy-2-piperidin-1-yl-[1,4]naphthoquinone



7k: 2-(1-Hydroxymethyl-propylamino)-5,8-dimethoxy-[1,4]naphthoquinone



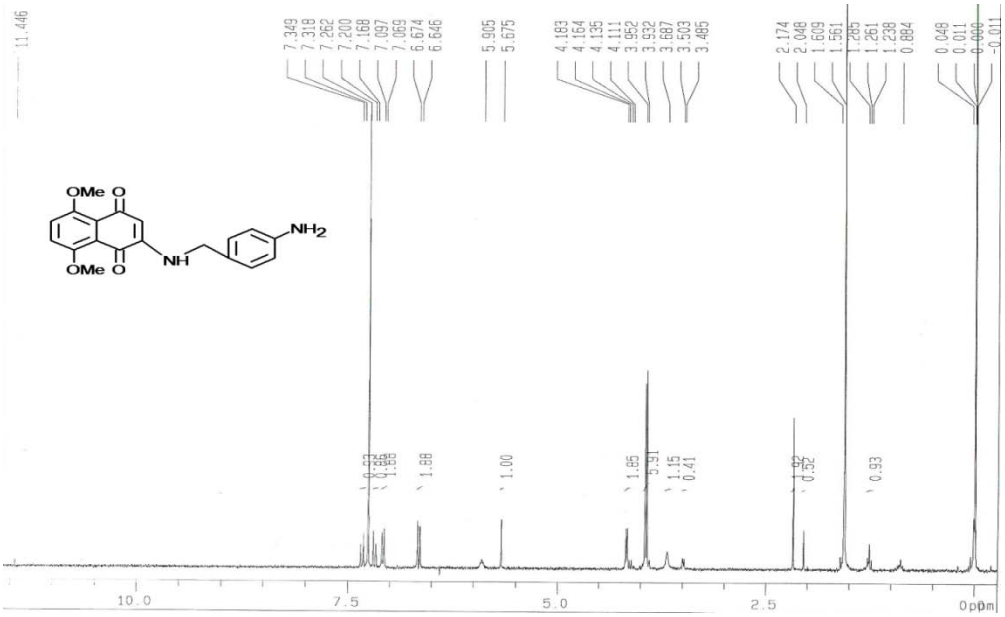
8k: 2-(2-Chloro-ethylamino)-5,8-dimethoxy-[1,4]naphthoquinone



9k: 2-(2,4-Dimethoxy-benzylamino)-5,8-dimethoxy-[1,4]naphthoquinone

10k: 4-(5,8-Dimethoxy-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-piperidine-1-carboxylic acid ethyl ester

11k: 4-[2-(5,8-Dimethoxy-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-ethyl]-benzeensulfonamide



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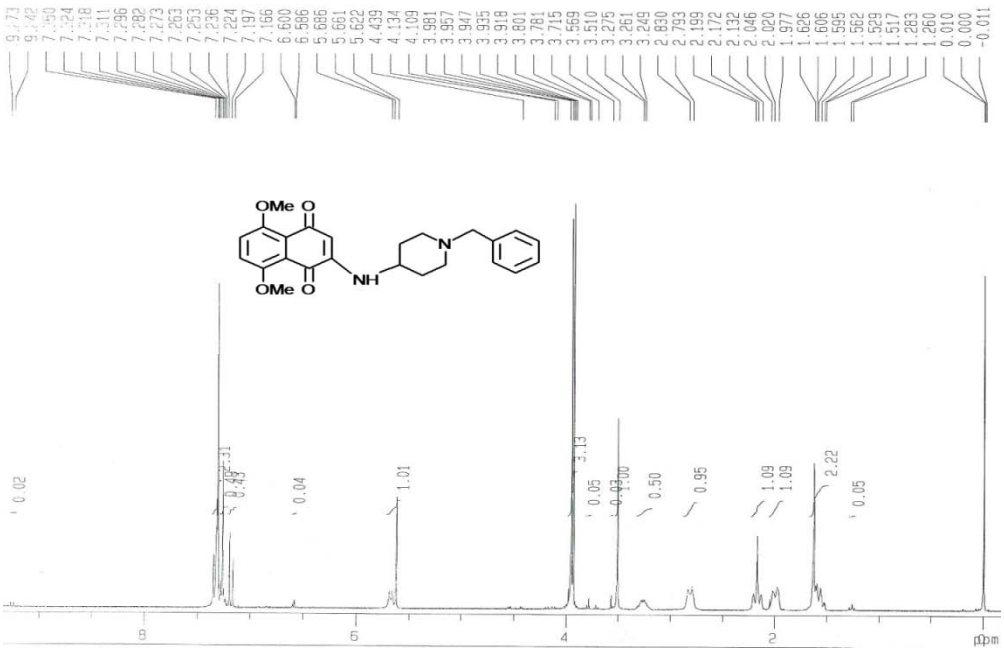

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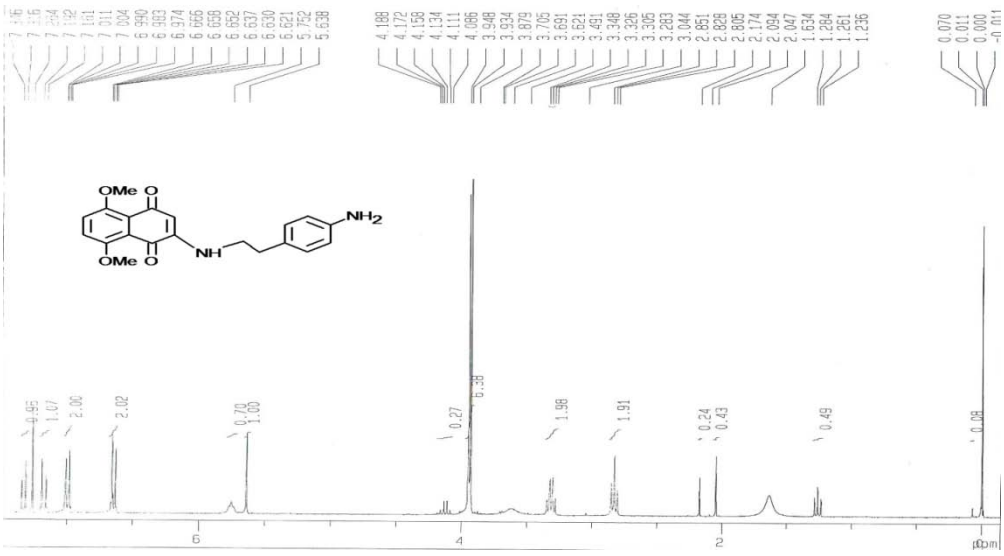
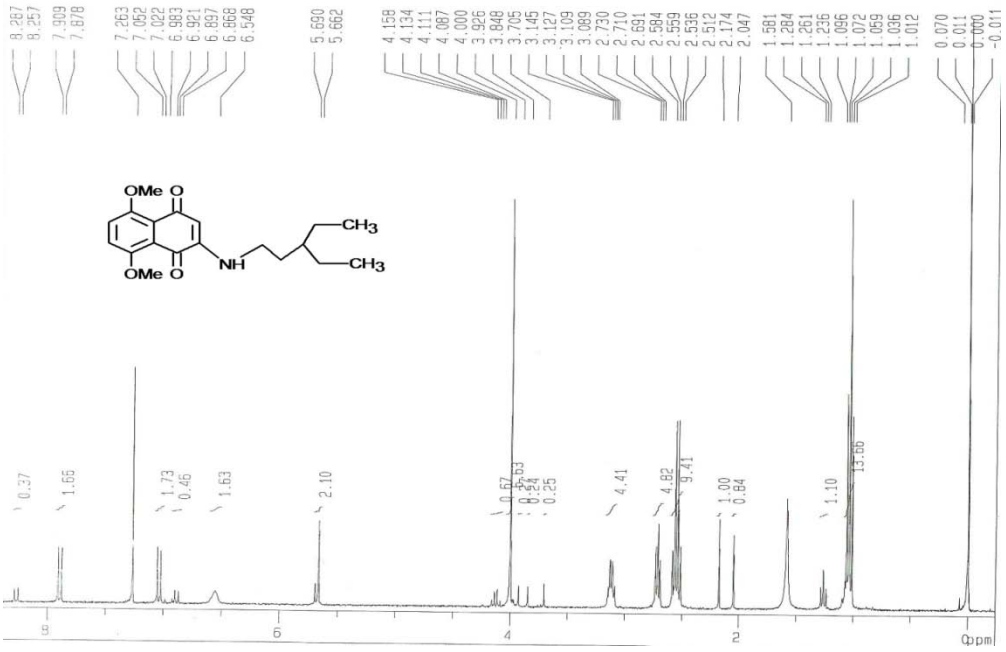
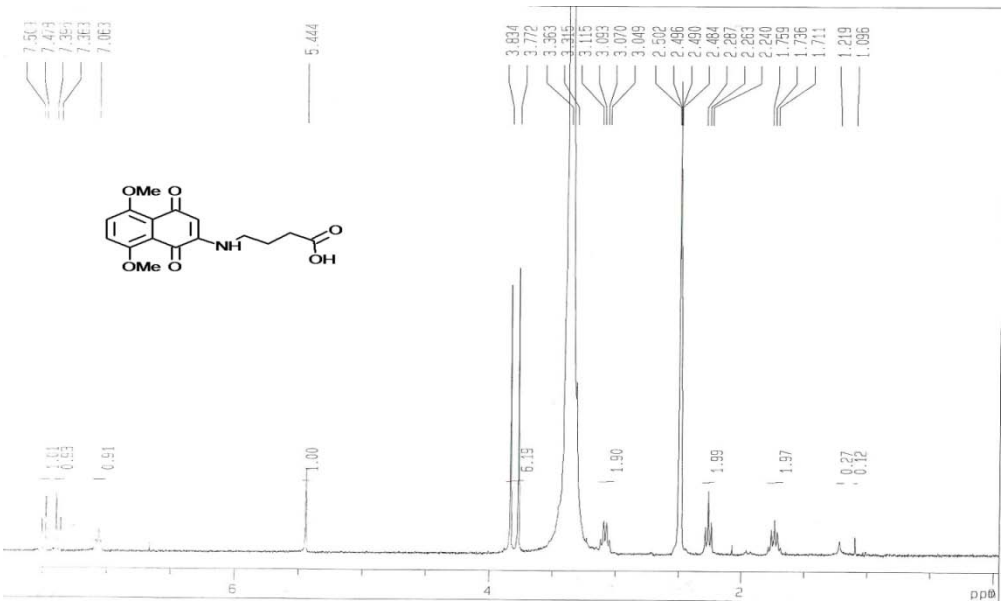


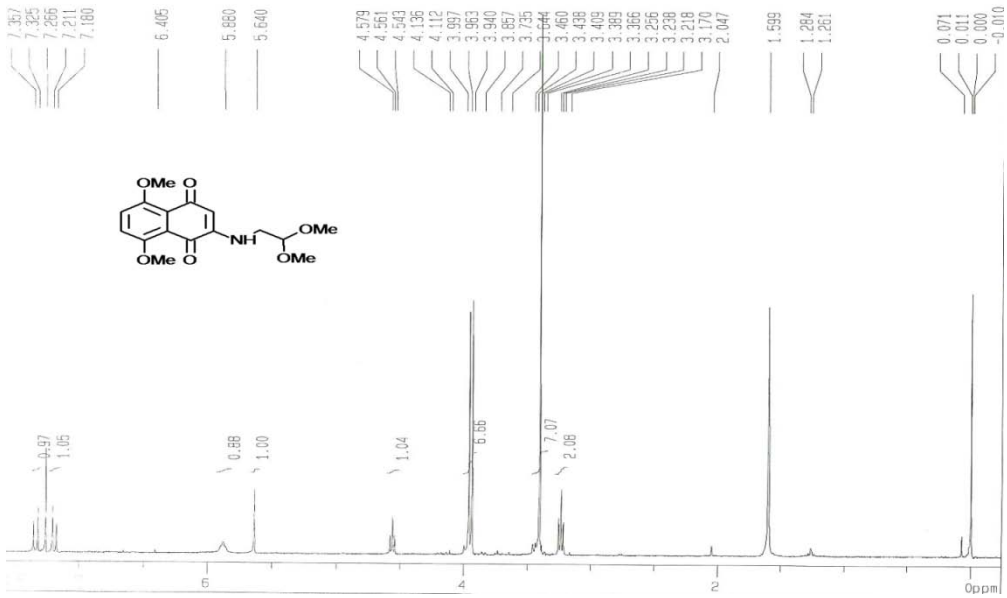
14k: 2-[2-(4-Amino-phenyl)-ethylamino]-5,8-dimethoxy-[1,4]naphthoquinone



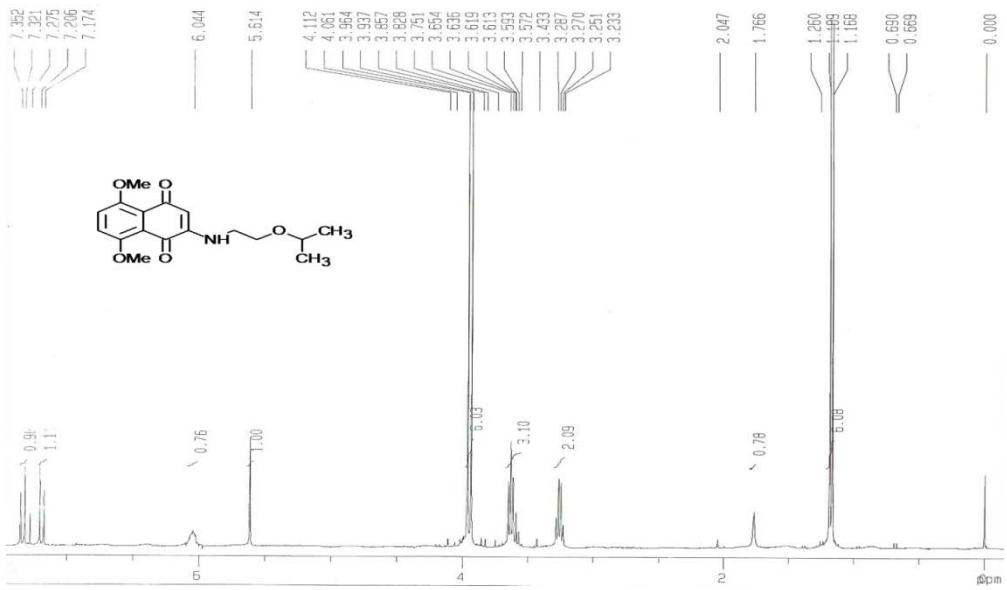
15k: 2-(2-Diethyamino-ethylamino)-5,8-dimethoxy-[1,4]naphthoquinone



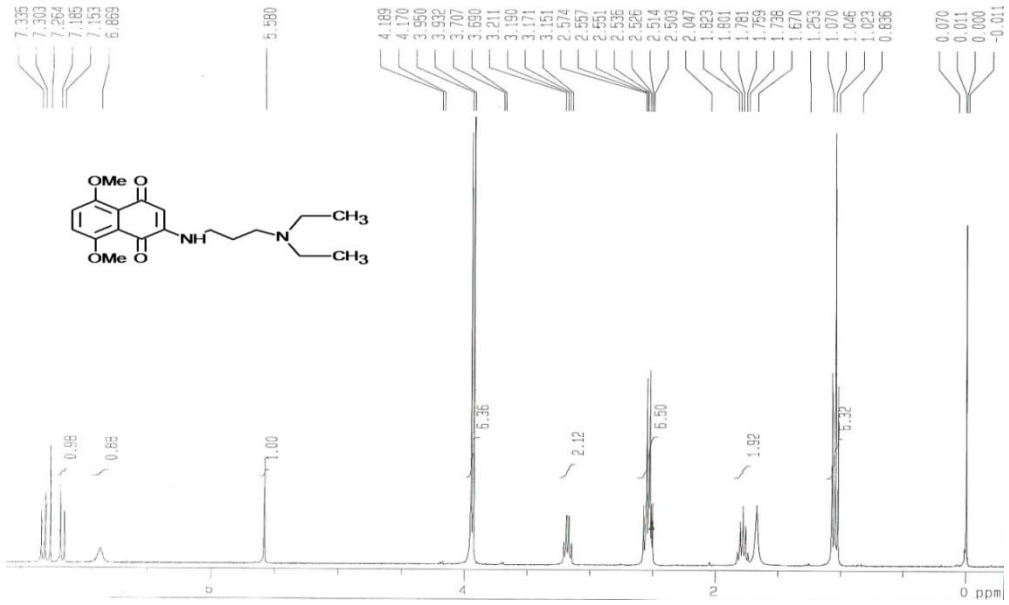
16k: 4-(5,8-Dimethoxy-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-butyric acid



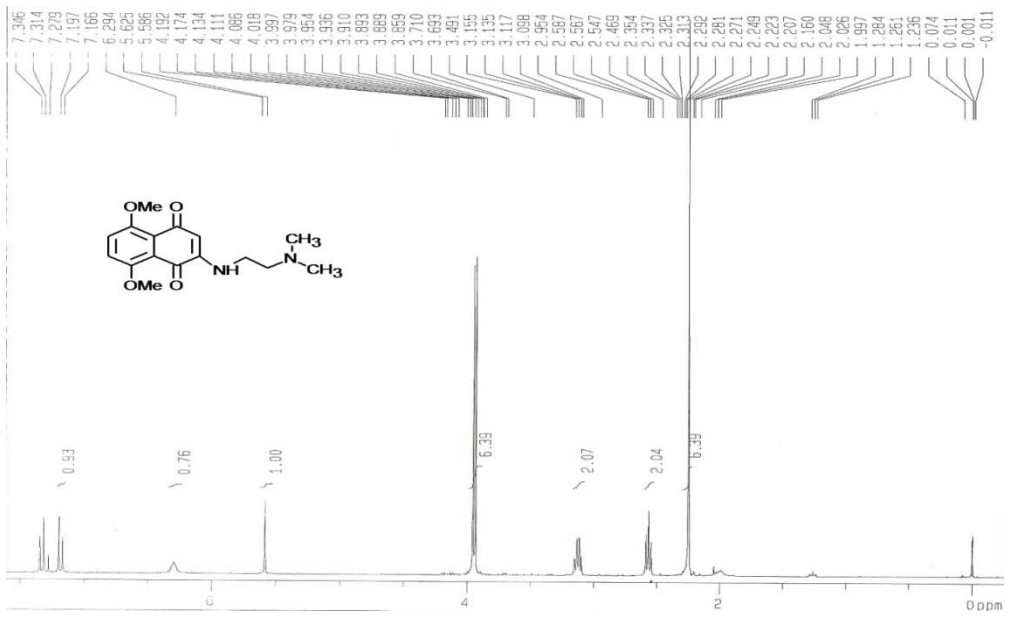
17k: 2-(2,2-Dimethoxy-ethylamino)-5,8-dimethoxy-[1,4]naphthoquinone



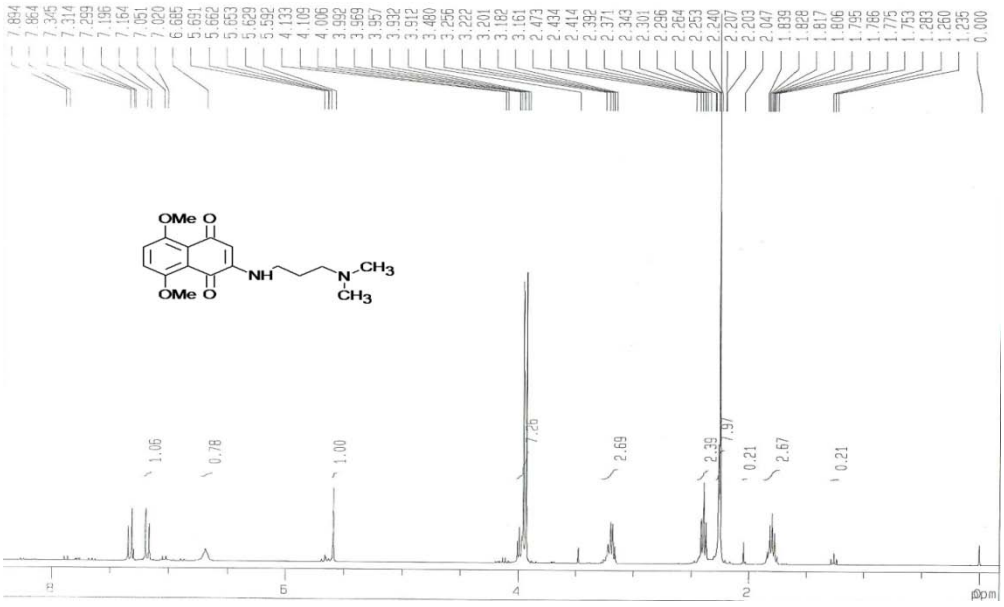
18k: 2-(2-Isopropoxy-ethylamino)-5,8-dimethoxy-[1,4]naphthoquinone



19k: 2-(3-Diethylamino-propylamino)-5,8-dimethoxy-[1,4]naphthoquinone

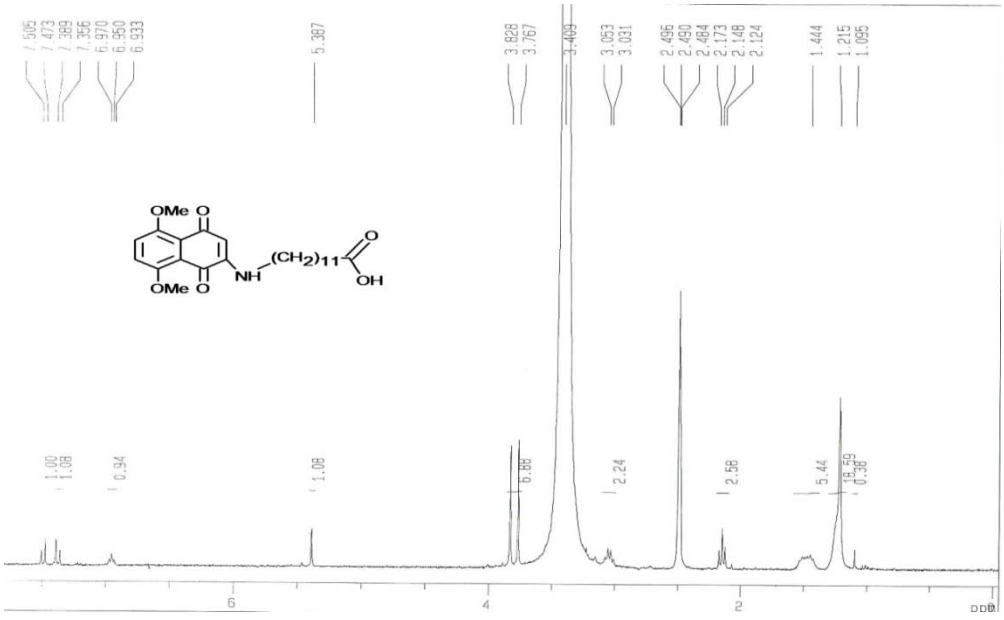


20k: 2-(2-Dimethylamino-ethylamino)-5,8-dimethoxy-[1,4]naphthoquinone

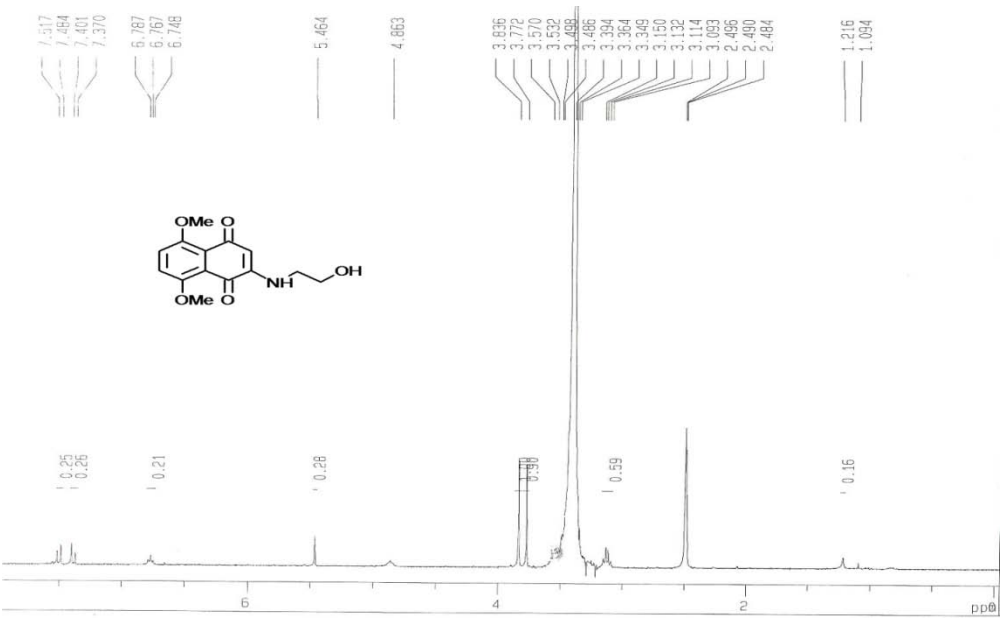


21k: 2-(3-Dimethylamino-propylamino)-5,8-dimethoxy-[1,4]naphthoquinone

22k: 5,8-Dimethoxy-2-(2-methylamino-ethylamino)-[1,4]naphthoquinone

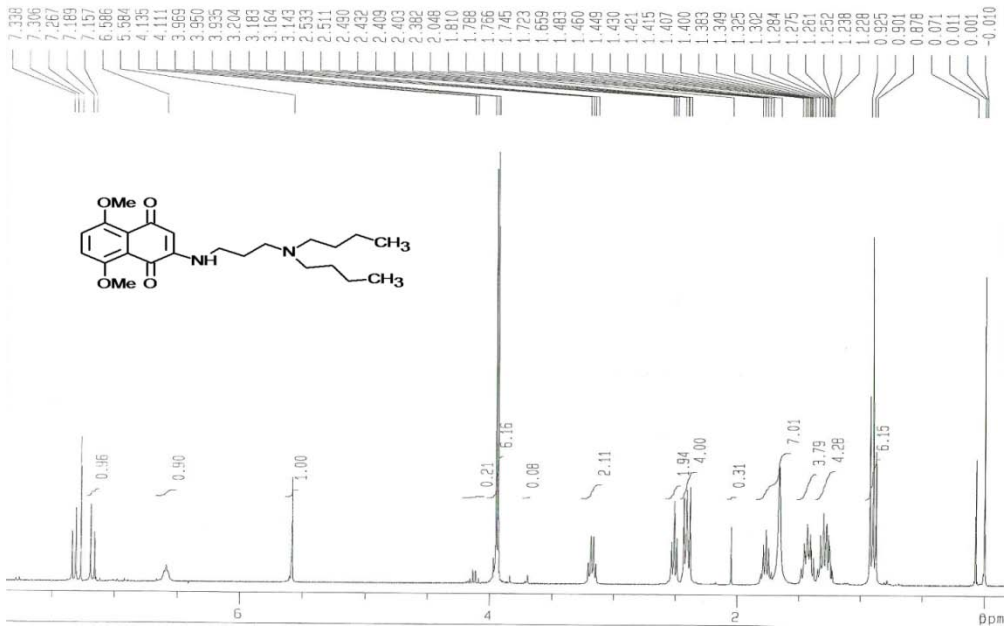


23k: 12-(5,8-Dimethoxy-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)dodecanoic acid

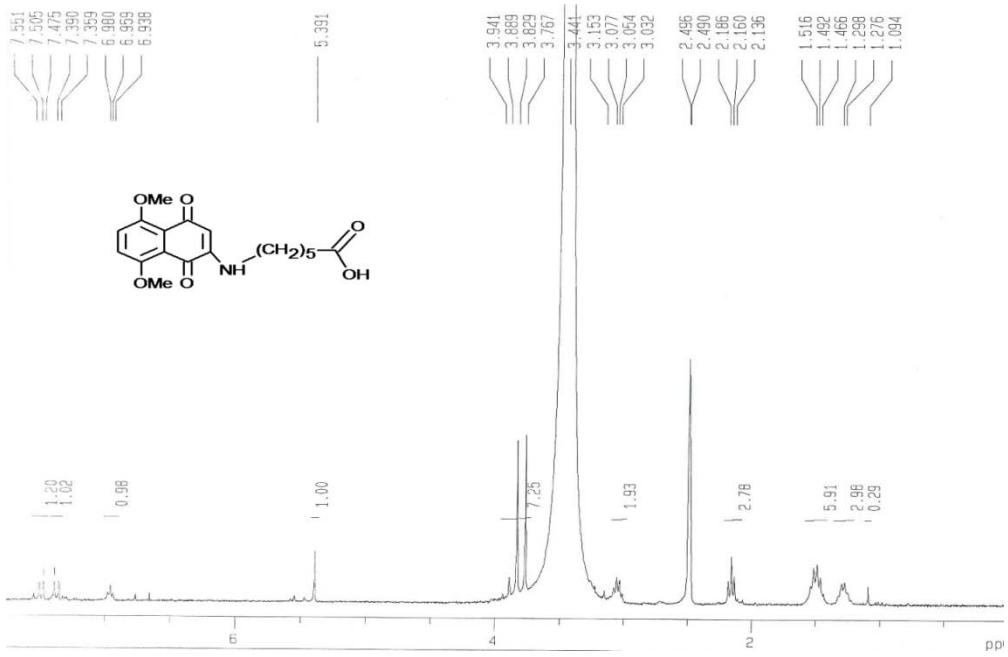


24k: 2-(2-Hydroxy-ethylamino)-5,8-dimethoxy-[1,4]naphthoquinone

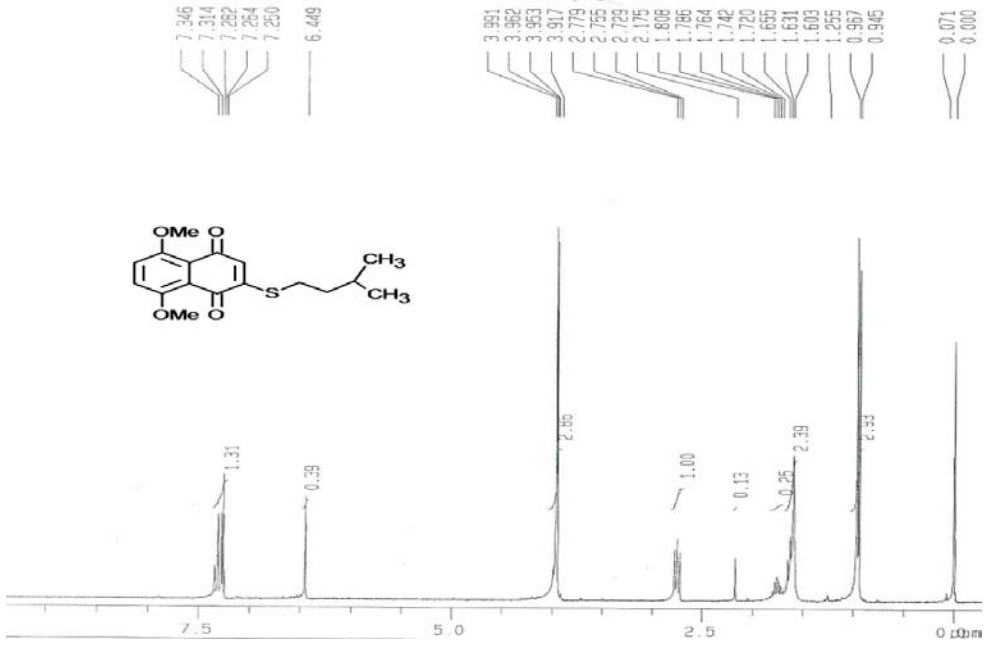
25k: 2-[2-(2-Hydroxy-ethoxy)-ethylamino]-5,8-dimethoxy-[1,4]naphthoquinone



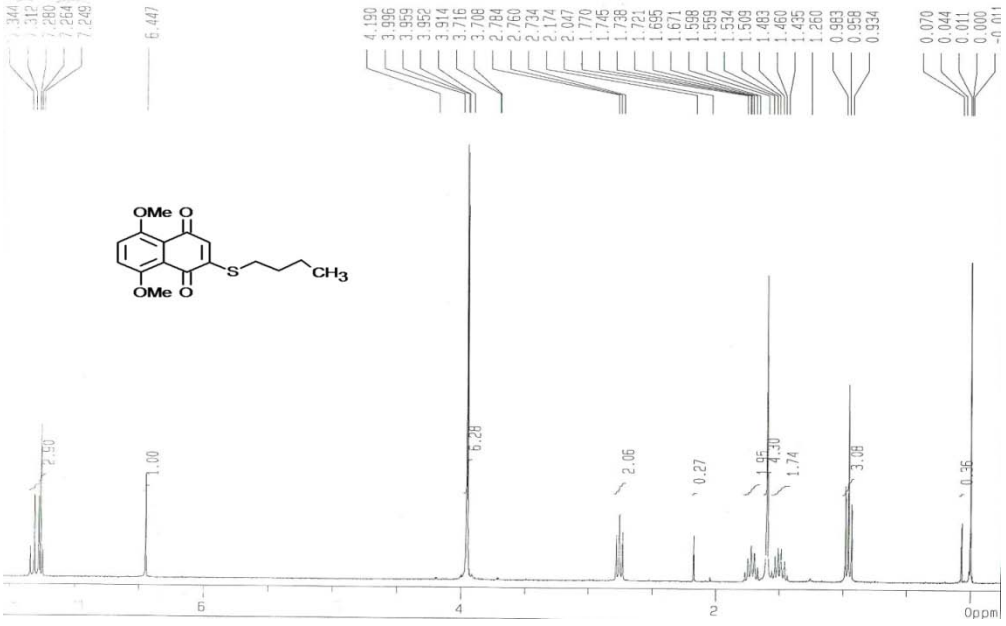
26k: 2-(3-Dibutylamino-propylamino)-5,8-dimethoxy-[1,4]naphthoquinone



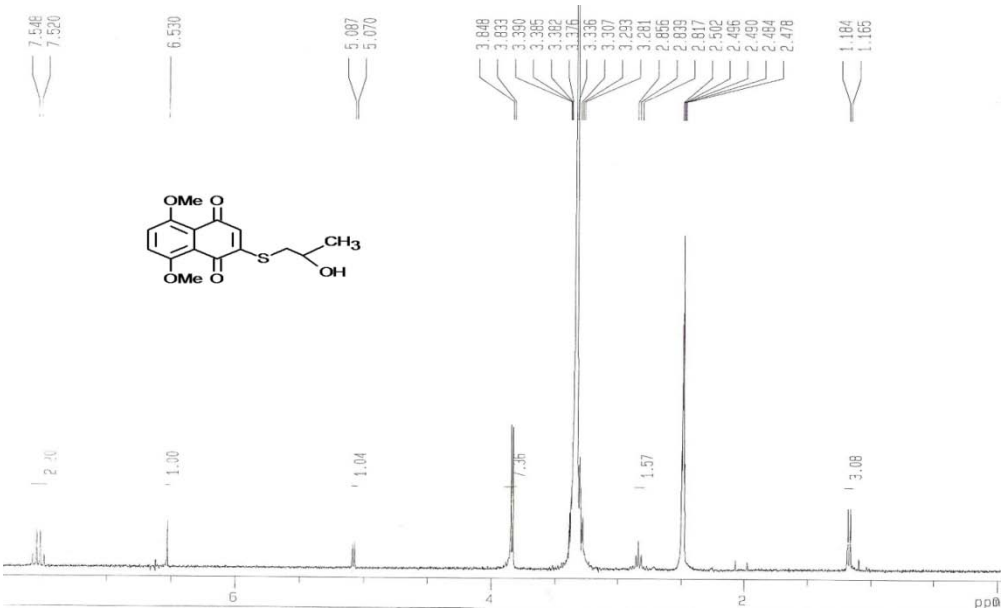
27k: 6-(5,8-Dimethoxy-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino)-hexanoic acid



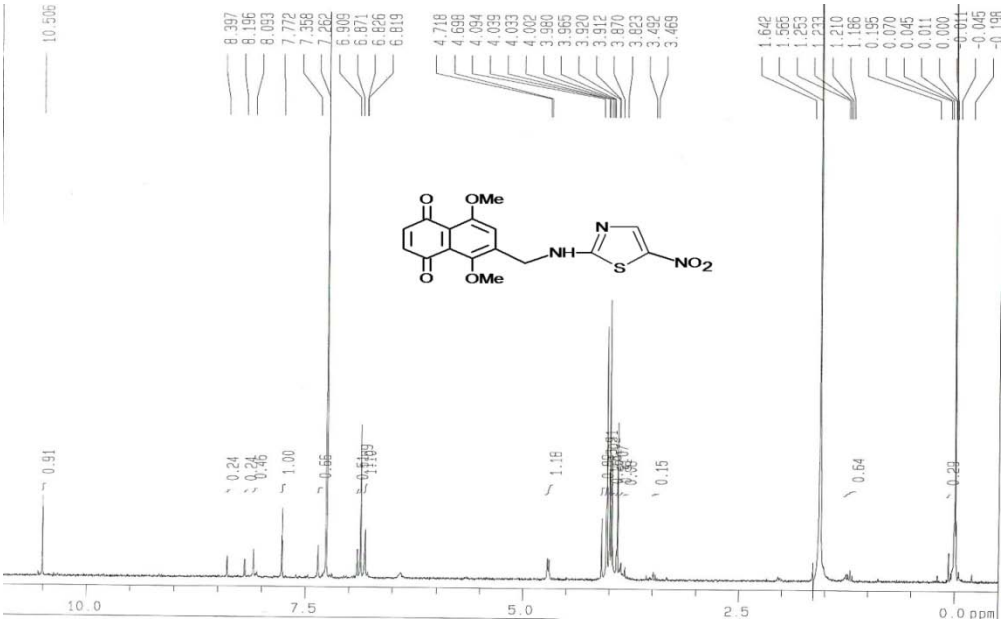
1q: 5,8-Dimethoxy-2-(3-methyl-butylsulfanyl)-[1,4]naphthoquinone



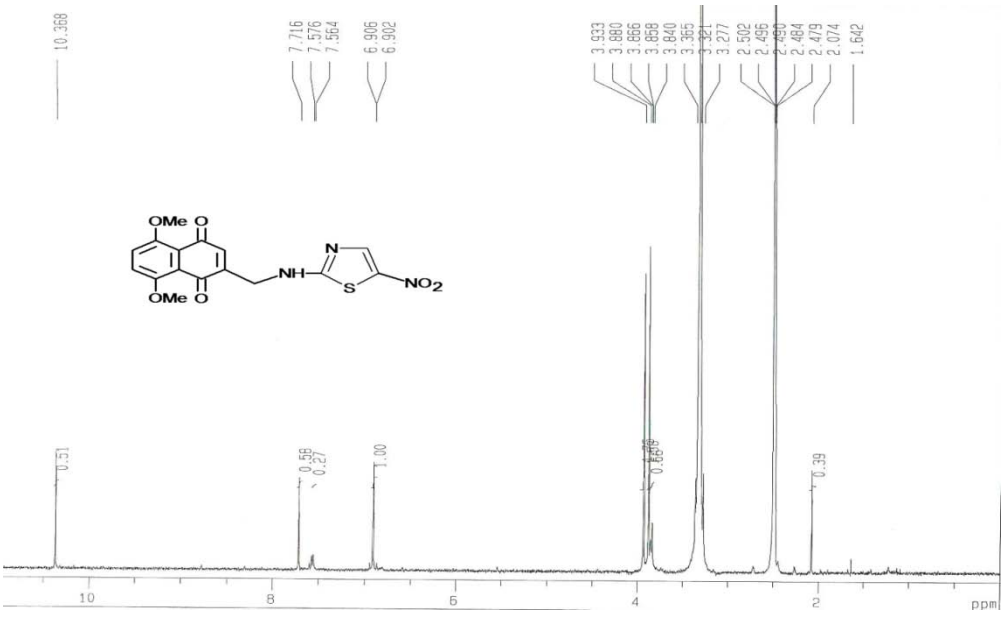
2q: 2-Butylsulfanyl-5,8-dimethoxy-[1,4]naphthoquinone



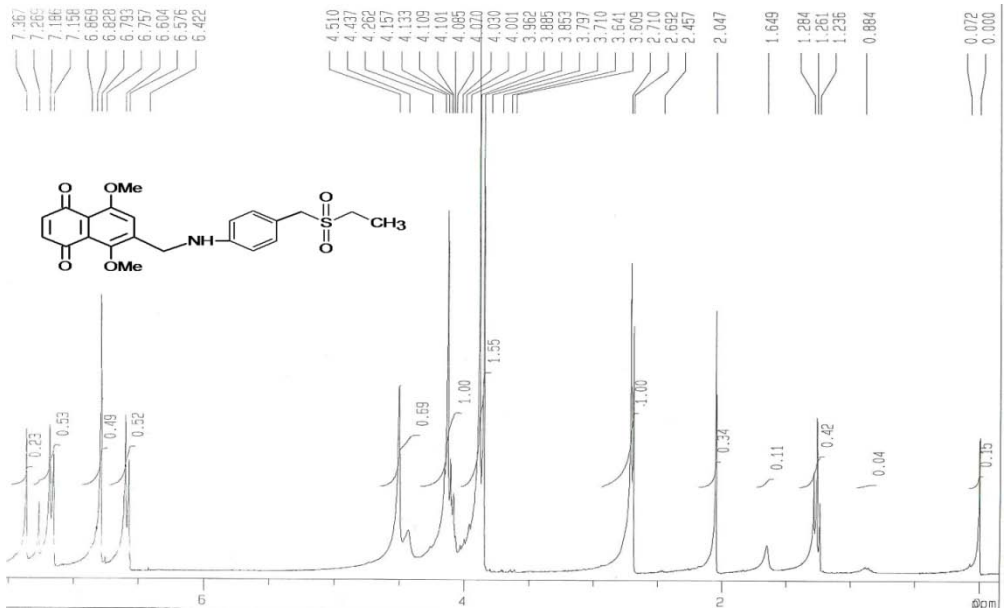
3q: 2-(2-Hydroxy-propylsulfanyl)-5,8-dimethoxy-[1,4]naphthoquinone



1c-6: 5,8-Dimethoxy-6-[(5-nitro-thiazol-2-ylamino)-methyl]-[1,4]naphthoquinone

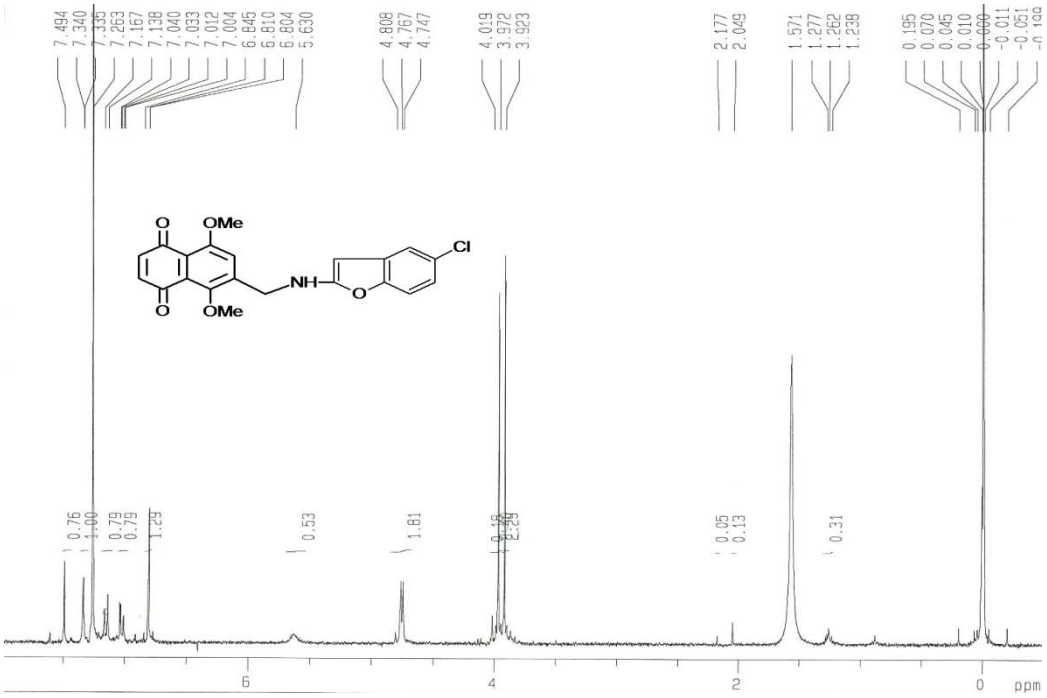


1c-2: 5,8-Dimethoxy-2-[(5-nitro-thiazol-2-ylamino)-methyl]-[1,4]naphthoquinone

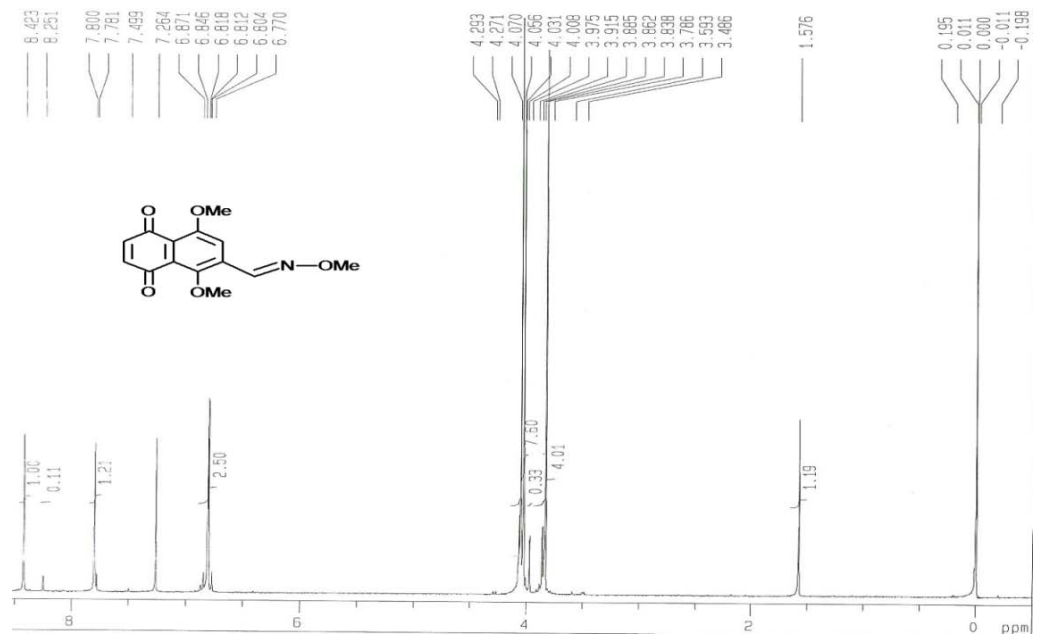


2c-6: *C*-{4-[(1,4-Dimethoxy-5,8-dioxo-5,8-dihydro-naphthalen-2-ylmethyl)-amino]-phenyl}-*N*-methyl-methanesulfonamide

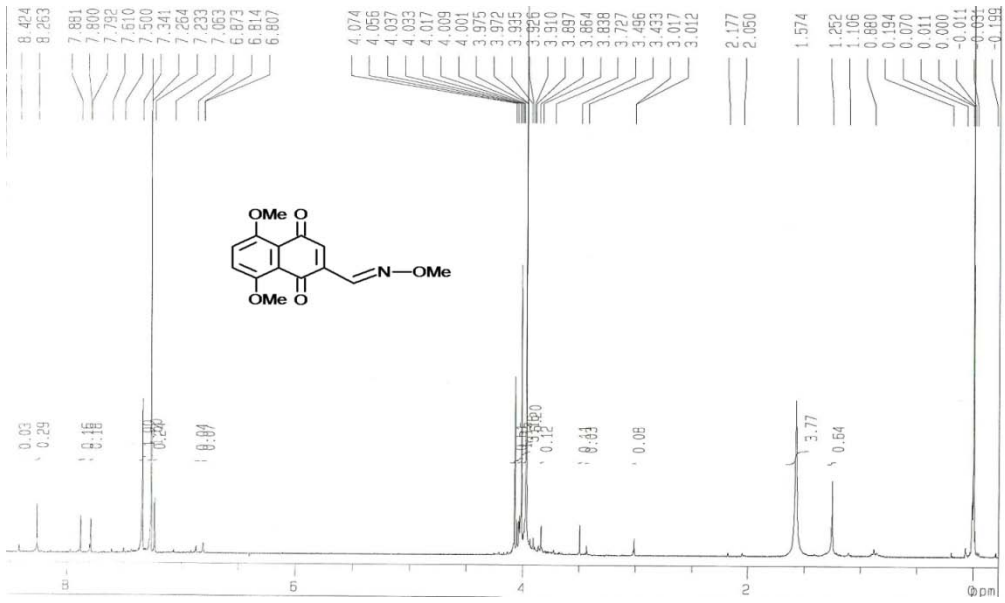
2c-2: *C*-{4-[(5,8-Dimethoxy-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl)-amino]-phenyl}-*N*-methyl-methanesulfonamide



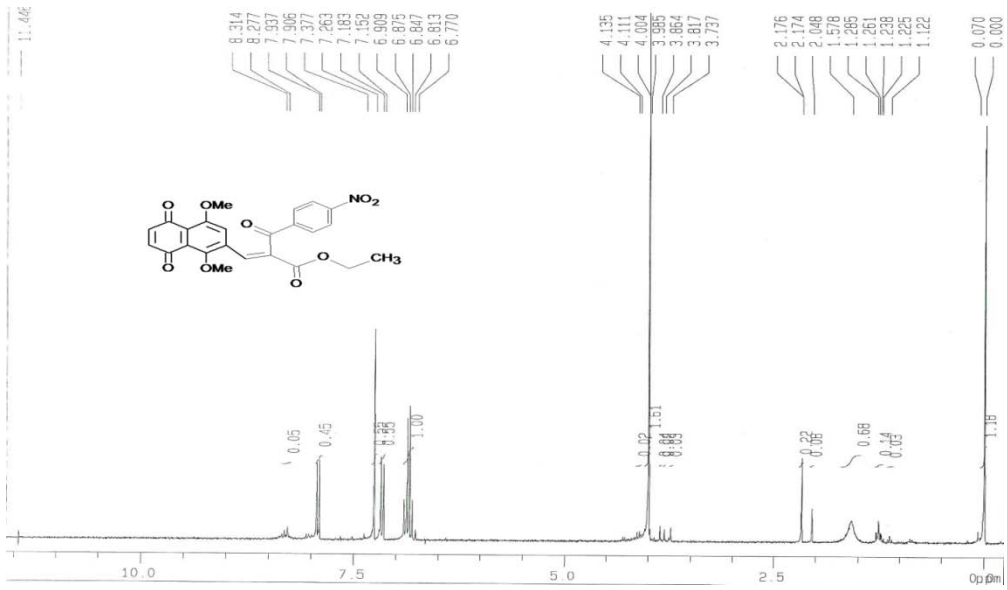
3c-6: 6-[(5-Chloro-benzooxazol-2-ylamino)-methyl]-5,8-dimethoxy-[1,4]naphthoquinone



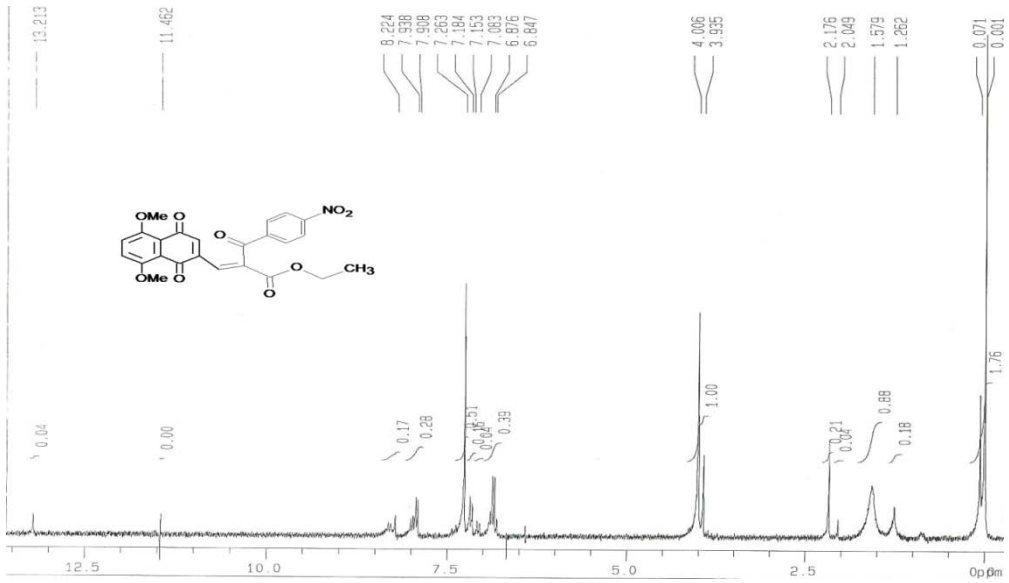
4c-6: 2-(Methoxyiminomethyl)-1,4-dimethoxy-5,8-dihydro-5,8-naphthalenedione



4c-2: 2-(Methoxyiminomethyl)-5,8-dimethoxy-1,4-dihydro-1,4-naphthalenedione



5c-6: Ethyl 2-(4-nitrobenzol)-3-(1,4-dimethoxy-5,8-dioxo-5,8-dihydro-2-naphthalenyl)-2-propenoate



5c-2: Ethyl 2-(4-nitrobenzol)-3-(5,8-dimethoxy-1,4-dioxo-1,4-dihydro-2-naphthalenyl)-2-propenoate

ACKNOWLEDGEMENT

With the deepest gratitude I wish to thank to whom I owe a great deal of their help and support to complete this thesis.

First of all, I would like to express my extreme gratitude to my advisor, Prof. Hoo Cho for his valuable support, encouragement, supervision, personal guidance, inspiration, and useful suggestions throughout the course of my research. For the time, I have learned helpful lessons from his experiences, knowledge, enormous enthusiasm as well as his wonderful kind personality.

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I also like to express my thanks to all my lab mates who co-worked in 2 years of my studying and helped me to adapt with new environment: Ms. Ying Wu, Ms. Yu-Lan Piao, Ms. A-Ram Song, Ms. So-Youn Lee, Ms. Eun-Jae Jang, Mr. Dea-Heung Byeun, Mr. Hyunj-Jun Kim, Mr. Truong Cong Chien.

I would like to give special thanks to my senior, Ms. Duong Thi Uyen who introduced me to Korea for studying, and I am also thankful to all my Vietnamese friends in Korea and Korean friends who helped me a lot in the 2 years. I also would like to express my sincere thanks to all Korean teachers in ABC centre who have taught me a lot Korean, and their supports.

I am extremely thankful to my parents and all my family for their love, support, teaching and encouragement in every moment of my life. From the bottom of my heart, I will ever always wish and pray for them.

저작물 이용 허락서

학 과	첨단부품소재공학과	학 번	20097752	과 정	석 사
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주 소	광주광역시 동구 서석동 조선대학교 공대 2 호관				
연락처	E-MAIL : hanglm87@yahoo.com				
논문제목	한글: 유해조류 제어를 위한 naphthoquinone 계열 살조물질 합성 및 구조활성 분석 영문: Synthesis and SAR of naphthoquinone as a novel class of algicides against harmful algal species				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB 구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집・형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포・전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5 년으로 하고, 기간종료 3 개월 이내에 별도의 의사표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1 개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송・출력을 허락함.

동의여부 : 동의(O) 반대( )

2011 년 8 월 25 일

작자: 레 티민 항 (서명)

조선대학교 총장 귀하