RESEARCH ARTICLE

New Amino-Anthracene-9, 10-Dione Derivatives: Synthesis and Pharmacological Evaluation as Powerful Neuroprotective and Antidepressant Agents

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ABSTRACT

The monoamine oxidase (MAO) enzyme resides in the outer mitochondrial membranes of all body cells, including the ones found in the brain, liver, and intestinal mucosa. Dopamine, serotonin, norepinephrine, tyramine, and tryptamine are extrinsic and indigenous amines that MAO oxidatively deaminates. MAO-A has been correlated with depression and other mental health issues, while MAO-B has been associated with the conditions Alzheimer's and Parkinson's disease. In a targeted assessment of naturally occurring anthraquinones, two isoforms of recombinant human MAOs were utilized. The inhibitory effects of purpurin and alizarin on MAO-A were observed, with calculated IC50 values of 2.50 and 30.1 M, respectively. This research on anthraquinones, purpurin, and alizarin offers promising new information that might eventually be used as a lead molecule in the discovery of the unique synthetic anthraquinones, anthracene 9, 10-dione compounds 1 to 9. On 96-well black polystyrene microtiter plates, both MAOs inhibiting action of 9 distinct synthetic anthracene 9,10-dione compounds has been evaluated. Compared to clorgyline, compounds 1, 2, 5, 8, and 9 inhibit MAO-A significantly, while compounds 1, 3, 5, 8, and 9 considerably inhibit MAO-B. Significant findings indicate that these compounds may be beneficial When employed to treat depression owing to their intense selective MAO-B activity and antidepressant effects as a result of their selective MAO-inhibition.

Keywords: Anthracene 9,10-dione, Neurological conditions, Parkinson's disease.

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INTRODUCTION

Due to natural anthraquinones' noteworthy abilities to inhibit MAO (both MAOs) enzymes and their antioxidant properties have garnered substantial attention in research. These attributes have been associated with potential antidepressant and neuroprotective effects. Among these anthraquinones, alizarin and purpurin (Figure 1), extracted from plants, exhibit the capacity to effectively inhibit the functionality of monoamine oxidase A and B (Table 1). Furthermore, their considerable antioxidant activity positions them as promising candidates for therapeutic interventions in neurological disorders like Alzheimer's and Parkinson's disease.[1]

The results of this investigation suggest that anthraquinones might be employed as antioxidants in the food and pharmaceutical sectors. It may be feasible to produce strong anthraquinones with neuroprotective and antidepressant characteristics by altering the original molecule with a range of heterocyclic chemicals, notably aromatic compounds with nitrogen. A number of investigations suggest that a heterocyclic nitrogen compound may inhibit MAO. Indole, pyrazoline, xanthine, oxadiazole, benzimidazole, pyrrole, quinoxaline, and thiazole are agents from the family of nitrogen heterocyclic MAO inhibitors that contain a carboxamide connection with amino-anthraquinone.[2] The importance of carboxamide linkage as a protective MAO inhibitory mechanism has been supported by several research. Using the proper synthetic methods, 1–9 molecules of 2-aminoanthraquinones, 1-aminoanthraquinones, and 1, 4-diaminoanthraquinones were produced. Considering their antioxidant and MAO inhibitory properties, anthraquinones

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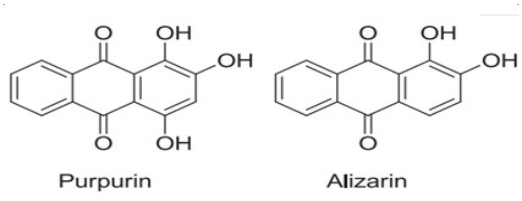


Figure 1: Purpurin and alizarin structures

Table 1: Purpurin and alizarin's respective IC_{50} values for inhibiting recombinant human MAOs

Anthraquinones	*IC_{50} (μM)*		*SI*
	MAO-A	*MAO-B*	
Purpurin	250 ± 0.35	≥40	≥16.0
Alizarin	30 ± 2.60	≥60	≥1.99

offer a potential alternative. In the investigation of MAO inhibition, human recombinant MAOs were employed. The experimental setup involved utilizing 96-well black polystyrene microtiter plates. Natural anthraquinones have demonstrated their value as lead compounds, guiding the development of novel synthetic molecules due to their established capability to hinder MAOs activity. Carboxamide linkages involving heterocyclic compounds have exhibited robust inhibitory effects on both MAOs. The synthesis of the new 1H-benzimidazole-anthraquinone-carboxamides 7–9 was accomplished through scheme 2, while the production of the 1H-pyrrole-anthraquinone-carboxamides 1–6 followed scheme 1.[3] These freshly synthesized compounds underwent testing to assess their potential in impeding the functions of monoamine oxidizing enzymes A and B.

MATERIALS AND METHODS

Synthesis of 1H-pyrrole-2-carbonyl chloride (A)

Below is a description of the steps involved in synthesizing 1,4-diaminoanthracene-9,10-dione and 1,1-aminoanthracene-9,10-dione derivatives using 1H-benzimidazole-2-carboxylic acid and 1H-pyrrole-2-carboxylic acid. All of the chemicals used were of analytical grade, including dichloromethane (DCM) from Loba Chemicals, 1,4-diaminoanthracene-9,10-dione, 1, H-pyrrole-2-carboxylic acid, and 1,H-benzimidazole-2-carboxylic acid from Sigma Aldrich (USA), NaH, and N,N-dimethylformamide (DMF) from TCA Chemicals.

In 1.25 g (7 mmol) of 1H-pyrrole-2-carboxylic acid and 2.5 mL (34 mmol) of thionyl chloride ($SOCl_2$) were mixed in 40 mL of dry dichloromethane (DCM) to create 1H-pyrrole-2-carbonyl chloride (A). For 6 hours, the mixture was reflux-stirred. The solution was then cooled to room temperature after the excess DCM and $SOCl_2$ were evaporated under reduced pressure. After being suspended in hexane, the resultant solid was evaporated to dryness, yielding 1.125 g (90%) of solid residue that was used exactly as-is.[4]

General Synthetic Procedure (Compounds 1-6)

- A solution of sodium hydride (NaH) (0.098 g, 3.9 mmol) in dried N,N-dimethylformamide (DMF) was mixed with 0.87 g (3.9 mmol) of a variety of substituted aminoanthraquinones. The mixture was stirred at room temperature for 30 minutes.
- The reaction mixture was then added 0.91 g (3.9 mmol) of 1H-pyrrole-2-carbonyl chloride and refluxed for 24 hours.
- The reaction mixture was refluxed before being cooled to room temperature. DMF was evaporated at a lower pressure, and the resultant residue was stirred in chloroform ($CHCl_3$) for ten minutes.
- The product was vacuum-dried after the solvent evaporated at reduced pressure.
- The final products were filtered and recrystallized to produce compounds 1-6 (Figure 2) (according to Scheme 1).

The strategy for synthesizing new compounds from 1,4-diaminoanthracene-9,10-dione and 1,aminoanthracene-9,10-dione coupled with 1H-pyrrole-2-carbonyl chloride is described above. The plan also includes how 1H-pyrrole-2-carbonyl chloride is made. The synthesis process for derivatives is outlined below.[5]

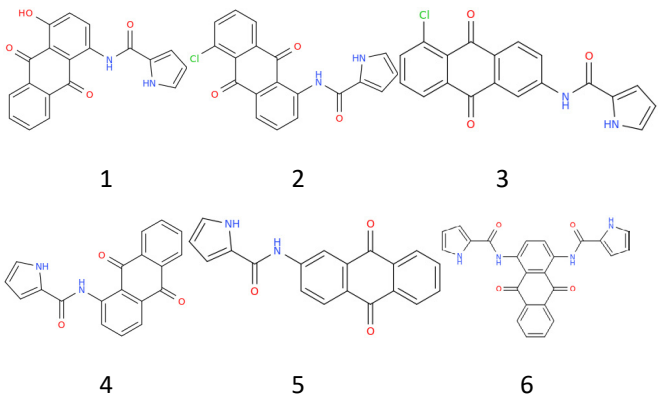
Preparation of 1-H-pyrrole-anthraquinone-carboxamide derivatives

Synthesis of 1H-benzimidazole-2-carbonyl chloride (B)

In 40 mL of dry dichloromethane (DCM) were used to dissolve 1.25 g (7 mmol) of 1H-benzimidazole-2-carboxylic acid and 2.5 mL (34 mmol) of thionyl chloride ($SOCl_2$). For six hours, the mixture was reflux-stirred. DCM and any residual $SOCl_2$ were refluxed, cooled to room temperature, and then evaporated under reduced pressure. The resultant solid residue was mixed with hexane, and the mixture was then evaporated to remove any moisture. This resulted in 1.062 g (85%) of solid residue, which was used immediately.[6,7]

General Synthetic Procedure Compounds

- Different substituted aminoanthraquinones were added to a sodium hydride (NaH) solution (0.098 g, 3.9 mmol) in dry N,N-dimethylformamide (DMF) at a rate of 0.87 g (3.9 mmol). The mixture was stirred at room temperature for 30 minutes.



Scheme 1: For synthesis 1-6 derivatives

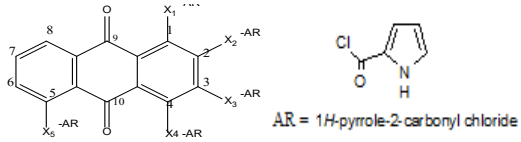
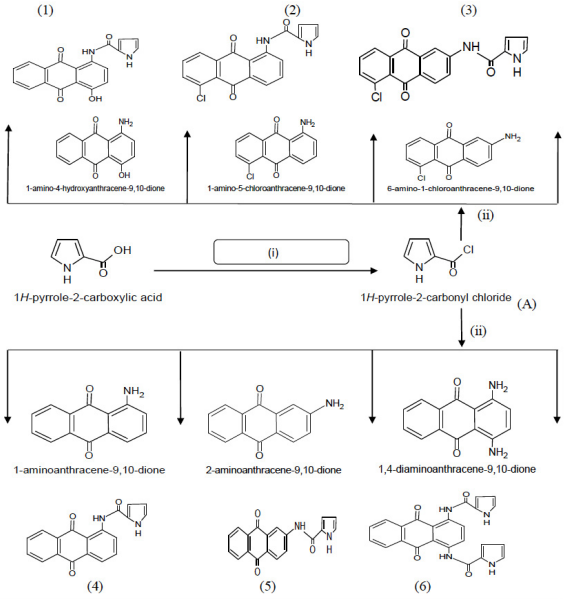


Figure 2: Novel Compounds of 1H-pyrrole-2-carbonyl chloride

Table 2: Compounds 1 to 6 alongwith all Functional Groups

Compounds	*X1-AR*	*X2-AR*	*X3-AR*	*X4-AR*	*X5-AR*
Comp 1	-NH_2-AR	-H	-H	-OH	-H
Comp 2	-NH_2-AR	-H	-H	-H	-Cl
Comp 3	-H	-NH_2-AR	-H	-H	-Cl
Comp 4	-NH_2-AR	-H	-H	-H	-H
Comp 5	-H	-NH_2-AR	-H	-H	-H
Comp 6	-NH_2-AR	-H	-H	-NH_2-AR	-H



Scheme 2: Synthesis route for the preparation of compounds (1-6). Reagents and conditions: (i) SOCl2, DCM/reflux, (ii) NaH, N, N-dimethylformamide, reflux 24h

- The reaction mixture was then given 0.91 g (3.9 mmol) of 1H-benzimidazole-2-carbonyl chloride and refluxed for 24 hours.
- The reaction mixture was cooled to room temperature following the reflux phase. The DMF was removed under reduced pressure, and the leftover material was stirred in chloroform ($CHCl_3$) for 10 minutes.
- The final product was dried under vacuum after the solvent evaporated under reduced pressure.
- The resulting materials were filtered and recrystallized to produce compounds 7-9 (Figure 3) (as shown in Scheme)

The above description outlines the procedure for synthesizing compounds 7-9 by linking various substituted aminoanthraquinones with 1H-benzimidazole-2-carbonyl chloride. The process also includes the synthesis of 1H-benzimidazole-2-carbonyl chloride itself. The specific steps involved in each stage of the synthesis are elaborated.[8,9]

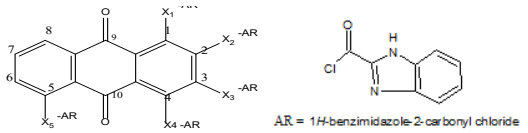
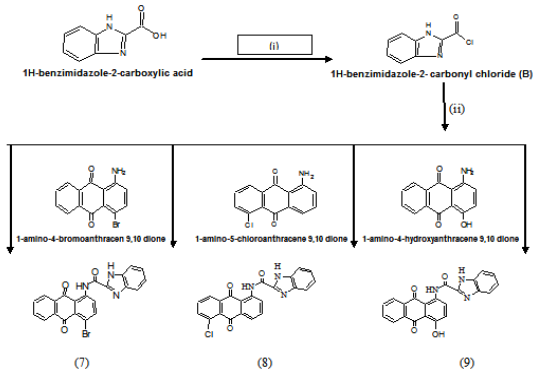


Figure 3: Novel Compounds of 1H-benzimidazole-2-carbonyl chloride

Table 3: Compounds 7 to 9 alongwith all Functional Groups

Compounds	*X1-AR*	*X2-AR*	*X3-AR*	*X4-AR*	*X5-AR*
Comp 7	-NH_2-AR	-H	-H	-Br	-H
Comp 8	-NH_2-AR	-H	-H	-H	-Cl
Comp 9	-NH_2-AR	-H	-H	-OH	-H



Scheme 3: Synthesis route for the preparation of compounds (7-9). Reagents and conditions: (1) SOCK, DCM/reflux (ii) NaH, N,N-dimethylformamide, reflux 24h

Preparation of 1H-benzimidazole-anthraquinone-carboxamide derivatives

A series of novel compounds derived from 1H-benzimidazole-anthraquinone-carboxamide (7-9), were successfully synthesized according to Scheme 2.

MAOS INHIBITORY ASSAY

Material and Methods

The following materials were employed: The supersomes TM were obtained from BD Gentest (United States), while all other compounds were procured from Sigma-Aldrich at analytical grade.

Procedure

Inhibition assays targeting MAOs were conducted using recombinant human enzymes. The measurements were executed within 96-well black polystyrene microtiter plates. The process comprised of pre-incubation, enzyme addition, incubation and reaction termination (Figures 4 and 5).

Control Measurements

Control groups were employed for comparative analysis. Specifically:

- For MAO-A inhibition: DMSO 1%, clorgyline 10^{-6} M (resulting in 100% inhibition), and pargyline 10^{-5} M (resulting in 100% inhibition) were used.
- DMSO 1% and pargyline 10^{-7} M (resulting in 50% inhibition) were employed for MAO-B inhibition.

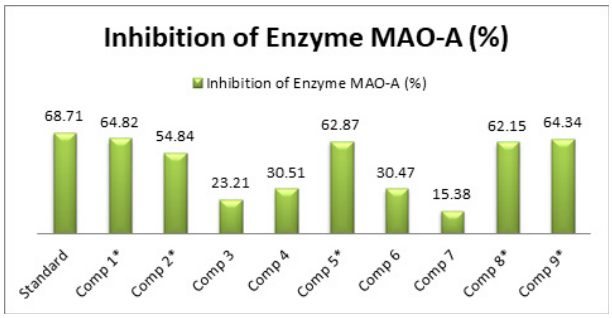


Figure 4: Impact of Synthetic Anthracenes on MAO - A

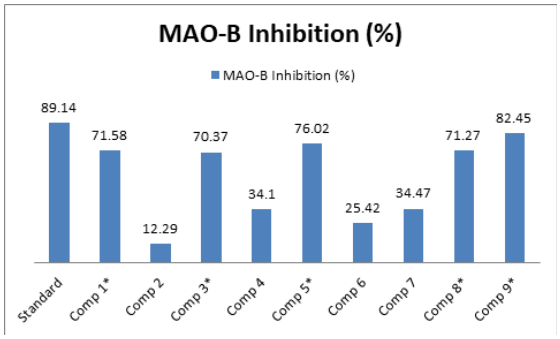


Figure 5: Effect of Synthetic Anthracenes on MAO - B

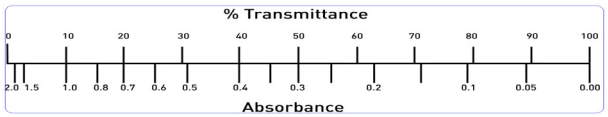


Figure 6: Optical density scale

Fluorescence Readings

Fluorescence readings were taken. This assay methodology was carried out to determine the inhibitory effects on MAOs enzymes using recombinant human enzymes. The precise steps, controls, and measurement parameters were meticulously followed in the process.[10]

Materials and Procedures (Assay)

The aforementioned resources were utilized. The supersomes TM were obtained from BD Gentest (United States), while all other compounds were procured from Sigma-Aldrich at analytical grade.

Procedure

Recombinant human enzymes were used in inhibition tests that targeted MAOs. Black polystyrene microtiter plates with 96 wells were used for the measurements. The steps in the Procedure were as follows:

- Pre-incubation: 158 L of potassium phosphate buffer (pH 7.4), 2 L of sample codes 1 to 9 (diluted in DMSO), and 20 L of Kynuramine solution (0.5 mM) were used to pre-incubate the microtiter plates. This pre-incubation took place at a temperature of 37°C for 20 minutes.
- Enzyme Addition: After the pre-incubation, 20 L of the enzyme solution were added to each well (0.09 mg/mL for MAO-A and 0.15 mg/mL for MAO-B).

Table 4: Effects of Compounds on MAO-A Inhibition

Group (100 µg/kg)	*Optical Density (OD)*	*Mean*	*MAO-A Inhibition (%)*
Clorgyline (MAO-A inhibitor)	0.135 0.139 0.129	0.134	67.39
Comp 1*	0.149 0.151 0.152	0.150	63.50
Comp 2*	0.189 0.192 0.193	0.191	53.52
Comp 3	0.321 0.324 0.320	0.321	21.89
Comp 4	0.289 0.291 0.293	0.291	29.19
Comp 5*	0.160 0.159 0.157	0.158	61.55
Comp 6	0.290 0.293 0.292	0.291	29.15
Comp 7	0.350 0.352 0.353	0.351	14.06
Comp 8*	0.160 0.162 0.161	0.161	60.83
Comp 9*	0.150 0.154 0.152	0.152	63.02

- Incubation: The wells that contained enzymes underwent a 30-minute incubation at a temperature of 37°C.
- Process Termination: 75 L of 1M NaOH was added to stop the enzymatic process after the incubation.

Control measurements: For comparative analysis, control groups were used. In particular: - DMSO 1%, Clorgyline 10^{-6} M (resulting in 100% inhibition), and Pargyline 10-5 M (resulting in 100% inhibition) were employed to inhibit MAO-A.

DMSO 1% and pargyline 10-7 M (providing a 50% inhibition) were used to suppress MAO-B.

Fluorescence measurements

Excitation and emission wavelengths used to measure fluorescence were 315 and 380 nm, respectively. Using recombinant human enzymes, this assay approach was used. The method was meticulously carried out according to the specified processes, controls, and measurement parameters.

Table 5: Findings of the impact of compounds on MAO-A Inhibition

Compounds	*Inhibition of enzyme MAO-A (%)*
Standard	68.71
1*	64.82
2*	54.84
3	23.21
4	30.51
5*	62.87
6	30.47
7	15.38
8*	62.15
9*	64.34

Table 6: Findings of the Impact of Compounds on MAO-B Inhibition

Compounds	*OD*	*Mean*	*Inhibition of enzyme MAO-A (%)*
	0.127		
Standard	0.119	0.120	87.87
	0.114		
	0.292		
1*	0.294	0.294	70.31
	0.296		
	0.880		
2	0.883	0.881	11.02
	0.882		
	0.305		
3*	0.304	0.305	69.10
	0.306		
	0.660		
4	0.666	0.665	32.83
	0.669		
	0.250		
5*	0.252	0.250	74.75
	0.248		
	0.748		
6	0.752	0.751	24.15
	0.754		
	0.659		
7	0.662	0.661	33.20
	0.664		
	0.296		
8*	0.297	0.297	70.00
	0.298		
	0.185		
9*	0.187	0.187	81.18
	0.189		

An optical density is defined by the following equation:

$$\text{Optical density} = \log\left(\frac{I_0}{I_t}\right)$$

I_0 = Incident light intensity
I_t = Transmitted light intensity

Table 7: Compounds inhibitory effects on MAO-B

Compounds	*MAO-B Inhibition (%)*
Standard	89.14
1*	71.58
2	12.29
3*	70.37
4	34.10
5*	76.02
6	25.42
7	34.47
8*	71.27
9*	82.45

Table 8: Physical properties of compounds

Compound	*Molecular Weight (g/mol)*	*Melting Point (°C)*	*Solubility*	*Appearance*
1	394.36	215–218	Soluble in DMSO, methanol	Yellow powder
2	412.81	178–180	Insoluble in water	Light brown crystals
3	412.81	191–193	Slightly soluble in ethanol	Orange solid
4	353.33	235–237	Soluble in DMF	Reddish-brown crystals
5	353.33	242–245	Insoluble in most solvents	Dark brown solid
6	482.46	168–170	Soluble in DMSO, Methanol	Dark green powder
7	497.18	187–190	Slightly soluble in ethanol	Pale yellow crystals
8	442.91	203–205	Soluble in DMF, DMSO	Light yellow solid
9	401.36	195–198	Insoluble in water	Pink powder

The optical density (OD) used in absorbance measurements is a logarithmic representation of the percent transmission (%T). A = log10 (100/%T) represents this relationship mathematically.

Absorbance values range from 0 (indicating 100% light transmitted) to 1 (indicating 10% light transmitted). This absorbance scale, spanning from 0 to 1.0, minimizes potential errors (Figure 6).

RESULTS AND DISCUSSION

Utilizing schemes 1 and 2, the following new compounds were produced.

Employing the previously outlined methodology for MAO inhibitory experiments using black polystyrene 96-well microtiter plates, an assessment was conducted on compounds

1-9 derived from the synthetic anthracene 9,10-dione. This evaluation aimed to ascertain their potential to inhibit both MAOs enzymes. Notably, all of the newly synthesized compounds, namely 1, 2, 5, 8, and 9, demonstrated significant MAO-A inhibitory effects when compared against the benchmark MAO-A inhibitor clorgyline. Furthermore, in a manner akin to the new synthetic compounds 1, 3, 5, 8, and 9, Pargyline serves as an MAO-B inhibitor. However, these novel molecules have displayed substantially enhanced effectiveness in the inhibition of MAO-B[11-13] (Tables 2 to 8).

CONCLUSION

The notable outcomes outlined above suggest that the innovative synthetic compounds 1, 3, 5, 8, and 9 can be utilized in addressing Parkinson's disease and other neurodegenerative conditions. This potential stems from their robust antioxidant properties and their discerning activity towards MAO-B. The recently synthesized anthracene 9,10-dione compounds, including 1, 2, 5, 8, and 9, exhibit considerable inhibition of MAO-A, rendering them promising contenders for mitigating depressive disorders.

Further exploration of these inventive synthetic compounds is warranted, as their implications could extend to the amelioration of depression and the manifestations associated with neurodegenerative ailments such as Parkinson's disease.

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