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Paper

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Synthesis of new anthraquinone compounds and evaluation of their considerable xanthine oxidase inhibitory activities

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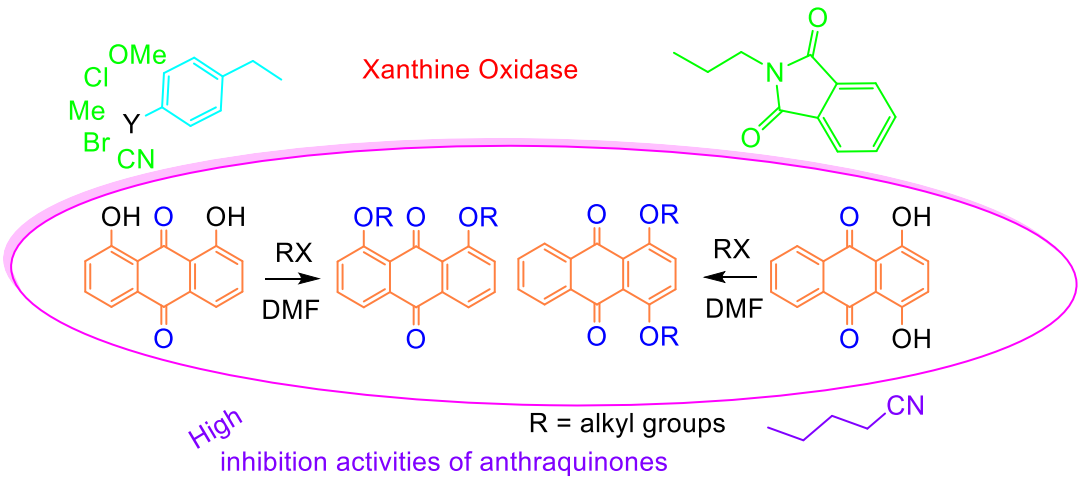
Abstract

A series of anthraquinone derivatives (**1-11**) was prepared using alkyl halides, quinizarin, and danthron. The structural analysis of new compounds was carried out by ^{1}H, ^{13}C NMR, IR, and UV-Vis spectroscopic techniques. Xanthine oxidase inhibitory activities of compounds were measured using allopurinol as a standard. Inhibition abilities were identified by measuring the uric acid formation at 294 nm at 37 °C. According to the IC_{50} values of the compounds, it was observed that all the compounds in the group had higher inhibition values than allopurinol.

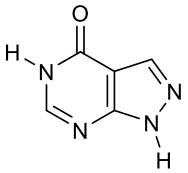


Keywords: Anthraquinone derivative, quinizarin, danthron, xanthine oxidase, inhibition

Introduction

Anthraquinones are important quinone derivatives of anthracene, which are both known to occur naturally in many plants and can be produced synthetically.[1-3] Anthraquinones have many application areas, especially in the paint industry, energy storage, medicinal chemistry, and sensor synthesis.[4-9] The most important advantages of anthraquinone dyes are their brightness and durability.[10-12] In addition to the above, anthraquinones have a wide spectrum of use in pharmaceutical chemistry. For example, anthraquinones are used as antineoplastic, anti-inflammatory, antiarthritic, antioxidant, antiviral, anticancer, antimicrobial, antitumor, mushil, and diuretic.[13-22] Also, anthraquinones are used in photodynamic therapy.[23,24]

Xanthine oxidase (XO) is an enzyme that acts as a catalyst during the degradation process of purines. In reaction, uric acid is formed from hypoxanthine and xanthine purine derivatives. Uric acid is a nitrogen-containing organic compound found in both animals and plants.[25-27] As is well known, the high uric acid level in the blood is called hyperuricemia, and the persistence of this condition leads to some diseases such as gout and kidney stone formation.[28-30] Also, due to activity of XO enzyme reason the formation of reactive oxygen species. The XO causes oxidative stress and then cancer formation with extreme activity in the long term.[31-33] In addition, XO inhibitor drugs are used to prevent such diseases. These drugs can be divided into two classes: purine analogs (allopurinol, oxypurinol, and tisopurine) and other (febuxostat, topiroxostat, and inositols) inhibitors. Febuxostat and topiroxostat are non-purine selective XO inhibitors.[34-39] The purine analog drug is named 1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one or allopurinol which was discovered in the 1950s and has been used as a drug for gout disease since 1966.[40]



Scheme. Structure of allopurinol.

Here, the newly synthesized anthraquinone derivatives were compared with allopurinol (scheme) as a standard. Because it is a well-known gout drug and structurally contains an oxo group similar to anthraquinones.

Results and Discussion

The Synthesis of compounds (1-11) was achieved using quinizarin (1,4-dihydroxyanthraquinone), chrysazin (1,8-dihydroxyanthraquinone), and alkyl halides with substitution reaction (Figure) apart from compound **5**. However, compound **5** was obtained by a Suzuki-Miyaura cross-coupling type reaction in the presence of a $Pd(OAc)_2/PPh_3$ catalyst. The yield of the compounds was calculated in the range of 51-79% and their melting points were measured between 153-273 °C. The structural evaluation for compounds was performed using ^1H, ^{13}C NMR, IR, and UV-Vis spectroscopic methods. The aromatic group peaks belonging to compounds were observed in the range of 8.26-6.93 ppm in the ^1H-NMR spectra. In addition, the chemical shift values of the benzylic CH_2 groups were observed at 5.19, 5.22, 5.20, 5.12, 5.33, 5.27, 5.44, 5.30, 5.32 ppm (for the

compounds **1**, **2**, **3**, **4**, **5**, **7**, **8**, **9**, and **10**) respectively. Also, peaks of quinone C=O groups between 183.5-181.9 ppm and benzylic peaks at the range of 71.9-69.8 ppm were observed in ^{13}C NMR spectra. Moreover, the peaks of C=O groups were observed at 1667, 1668, 1665, 1666, 1668, 1664, 1666, 1668, 1666, 1668, and 1676 cm-1, respectively. The stretching vibrations values of the CN groups in compounds 3, 6, and 8 were determined as 2227, 2241, and 2228 cm-1. These values are in agreement with the literature.[41]

The UV-Visible spectral analysis also was performed for anthraquinone derivatives. The lower band (280-364 nm) in the electronic spectra of anthraquinones corresponds to π → σ* transitions. The π → π* transitions were observed in the visible area for compounds at λ_{max} = 485, 473, 477, 473, 435, 472, 486, 476, 487, 481, and 494 nm respectively. The data are compatible with the literature data.[42]

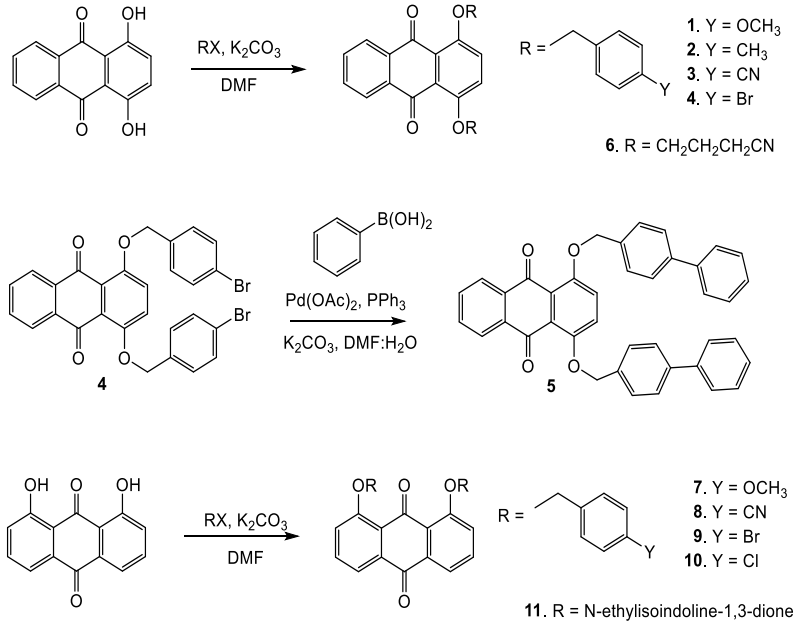


Figure. Synthesis procedure of new anthraquinones.

There are synthetic or natural many drugs that have an anthraquinone core. For example, rhein has a kidney protective effect; emodin is known to have antitumoral, antimicrobial, and antioxidant effects, as well as a protective effect on the liver and kidneys. In addition, chrysophanol and physcion have a protective effect on the nervous system.[43,44]

In a study recorded in 1987, the XO inhibition effect of nine anthraquinone compounds was observed in vitro by Noro et al. Here, it was recorded that quinizarin and chrysazine had weak inhibitory activity when compared with other anthraquinone derivatives.[45]

In this work, the XO enzyme inhibition abilities were tested of the novel quinizarin and danthron alkoxy derivatives, and their IC_{50} values were determined between 0.339-1.015 µM (Table). IC_{50} is used to mean half-maximal inhibitory concentration. It is the most common measure of the effectiveness of a drug. IC50 values indicate the essential amount of inhibitor for half-inhibition to occur. If the IC50 value is smaller, it is the more effective inhibitor. The IC_{50} value of allopurinol, which is used as a standard drug, was observed as 1.967 µM. Therefore, it is understood that all anthraquinone compounds have a higher inhibition effect than allopurinol. The IC_{50} values of compounds **3** and **4** (1,4-bis(4-cyanobenzyl)oxy)anthracene-9,10-dione and 1,4-bis(4-bromobenzyl)oxy)anthracene-9,10-dione) are 0.339 and 0.395 µM. But the inhibition values of 1,8-disubstituted anthraquinone derivatives with the same functional groups (compounds **8** and **9**) were

measured as 0.803 and 0.748 µM. These values show that quinizarin derivatives with the same functional groups have higher inhibitory power than danthron derivatives. Also, the structure of ethylisoindoline-1,3-dione used for the synthesis of compound **11** contains two oxo groups, it was used to observe in which direction the inhibitory activity of the compound would change. It was seen from the measurement results that compound **11** has one of the highest activity (IC_{50} **=** 0.365 µM).

In previous studies, XO inhibition activities of some groups of compounds were reported as IC_{50} for XO; such as pyridine salts between 0,394-0,623 µM [46], N-heterocyclic carbene ligands between 0.58-1.69 µM [47], and new pyrrole carboxamide derivatives between 4.608-7.084 µM.[48] When the IC_{50} values of the inhibitory agents in the mentioned literature sources are compared with the anthraquinones, it is understood that the new anthraquinone derivatives are more remarkable inhibitors than other compound groups, because of IC50 values (0.339, 0.365, and 0.395 µM). Due to these results, the most active compounds (**3**, **4**, and **11**) can be an alternative to allopurinol.

Table. The inhibition values (IC_{50}) of the anthraquinones on xanthine oxidase enzyme (XO)

Compound	IC_{50} (µM)	r^2
1	0.837 ± 0.045	0.991
2	0.567 ± 0.073	0.964
3	0.339 ± 0.029	0.992
4	0.395 ± 0.015	0.971
5	1.015 ± 0.061	0.956
6	0.401 ± 0.011	0.977
7	0.641 ± 0.024	0.982
8	0.803 ± 0.061	0.973
9	0.748 ± 0.016	0.971
10	0.947 ± 0.058	0.951
11	0.365 ± 0.011	0.917
Allopurinol	1.967 ± 0.082	0.979

Conclusions

Briefly, in this latest study, 11 new anthraquinone derivatives were synthesized from 1,4-dihydroxyanthraquinone and 1,8-dihydroxyanthraquinone and their structures were determined by spectroscopic methods. In the continuation of the study, the xanthine oxidase inhibition activities of the compounds were tested and compared with the standard drug, allopurinol. All compounds showed higher inhibitory activity than allopurinol. Moreover, it appears that four compounds; 1,4-bis(4-cyanobenzyl)oxy)anthracene-9,10-dione, 1,4-bis(4-bromobenzyl)oxy)anthracene-9,10-dione, 1,4-Bis(3-cyanopropyl)oxy)anthracene-9,10-dione, and 2,2'-(((9,10-dioxo-9,10-dihydroanthracene-1,4-diyl)bis(oxy))bis(ethane-2,1-diyl))bis(isoindoline-1,3-dione) (**3**,**4**, **6** and **11**) have higher inhibition activity than others respectively. On the other hand, compound **5** was observed to have the least activity against the XO enzyme in the series. The above mentioned compounds are encouraging for new lead structures for further drug development.

Experimental Section

General conditions and materials. The reactions were performed in atmospheric conditions. Starting materials and reagents used in reactions were supplied commercially from Aldrich or Merck Chemical Co. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded using a Bruker Avenced III 400 MHz FT NMR spectrometer. Infrared (IR) spectra were recorded using an ATR unit from 4000 to 200 cm^{-1} on a Perkin-Elmer FT-IR spectrophotometer. Elemental analyses were performed with a LECO CHNS-932 elemental analyzer. UV-Vis spectra were measured on a Perkin-Elmer Lambda 35 spectrophotometer. Melting points were recorded using an electrothermal-9200 melting point apparatus and are uncorrected.

General synthesis procedure

Anthraquinone derivatives (**1-4**, **6-11**) were synthesized from 1,4-dihydroxyanthraquinone, 1,8-dihydroxyanthraquinone and alkyl halides in *N,N*-dimethylformamide (DMF) in the presence of K_2CO_3. Unlike other compounds, compound **5** was obtained as a result of the C-C coupling reaction of compound **4** and benzeneboronic acid in presence of a catalyst system consisting of $Pd(OAc)_2/PPh_3$. (Figure).

Synthesis of 1,4-Bis(4-methoxybenzyl)oxy)anthracene-9,10-dione (1). A mixture of 1,4-dihydroxyanthraquinone (1.00 g, 4.17 mmol), 4-methoxybenzyl chloride (1.31 g, 8.34 mmol), K_2CO_3 (1.15 g, 8.34 mmol) in DMF (20 mL) was heated by reflux in a water bath for 12 hours. The color of the reaction mixture changed from orange to yellow. At the end of the reaction, the mixture in the bottom flask was poured into cold water and precipitated. The yellow solid crude product was crystallized from an ethanol/chloroform (1:1) mixture after washing three times with water. A yellow-colored compound was obtained. Yield (%): 75 (1.50 g), mp 192-193 °C. Anal Calcd (%) for $C_{30}H_{24}O_6$ (480.2 g/mol): C 74.99, H 5.03. Found (%): C 74.52, H 5.01. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.06-8.04 (*m*, 2H, Ar-H), 7.84-7.82 (*m*, 2H, Ar-H), 7.63 (*s*, 2H, Ar-H), 7.52 (*d*, 4H, Ar-H, *J* 8 Hz), 6.99 (*d*, 4H, Ar-H, *J* 8 Hz), 5.19 (*s*, 4H, CH_2), 3.78 (*s*, 6H, OCH_3) ppm. ^{13}C-NMR ($CDCl_3$, 100 MHz): 182.6 (C=O), 159.4, 153.1, 134.2, 194.0, 129.4, 129.2, 126.4, 123.4, 123.0, 114.2 (Ar-C), 71.0 (CH_2), 55.6 (OCH_3) ppm. IR: 1667 $\nu_{(C=O)}$, 1513 $\nu_{(quinone)}$, 1237 cm^{-1} $\nu_{(arom.\ CH)}$.

Other anthraquinone derivatives (**2-4**,**6-11**) were synthesized using 1,4-dihydroxyanthraquinone, 1,8-dihydroxyanthraquinone, and related alkyl halides by a similar process.

1,4-Bis(4-methylbenzyl)oxy)anthracene-9,10-dione (2). Yield (%): 71 (1.32 g), mp 186-187 °C. Anal Calcd (%) for $C_{30}H_{24}O_4$ (448.5 g/mol): C 80.34, H 5.39. Found (%): C 80.07, H 5.22. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.07-8.05 (*m*, 2H, Ar-H), 7.84-7.82 (*m*, 2H, Ar-H), 7.62 (*s*, 2H, Ar-H), 7.48 (*d*, 4H, Ar-H, *J 8* Hz), 7.23 (*d*, 4H, Ar-H, *J* 8 Hz), 5.22 (*s*, 4H, CH_2), 2.33 (*s*, 6H, CH_3) ppm. ^{13}C-NMR ($CDCl_3$, 100 MHz): 182.6 (C=O), 153.1, 137.3, 134.4, 134.2, 134.0, 129.4, 127.8, 126.4, 123.3, 122.9 (Ar-C), 71.1 (CH_2), 21.3 (CH_3) ppm. IR: 1668 $\nu_{(C=O)}$, 1589 $\nu_{(kinon)}$, 1235 cm^{-1} $\nu_{(arom.\ CH)}$.

1,4-Bis(4-cyanobenzyl)oxy)anthracene-9,10-dione (3). Yield (%): 74 (1.45 g), mp 227-228 °C. Anal Calcd (%) for $C_{30}H_{18}N_2O_4$ (470.5 g/mol): C 76.59, H 3.86, N 5.95. Found (%): C 76.27, H 3.72, N 5.55. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.14-8.12 (*m*, 2H, Ar-H), 7.72-7.65 (*m*, 10H, Ar-H), 7.23-7.19 (*m*, 2H, Ar-H), 5.20 (*s*, 4H, CH_2) ppm. ^{13}C-NMR ($CDCl_3$, 100 MHz): 182.9 (C=O), 153.1, 141.8, 134.0, 133.5, 132.5, 127.4, 126.6, 124.1, 122.1, 111.8 (Ar-C), 118.8 (CN), 70.9 (CH_2) ppm. IR: 2227 $\nu_{(CN)}$, 1665 $\nu_{(C=O)}$, 1566 $\nu_{(quinone)}$, 1243 cm^{-1} $\nu_{(arom.\ CH)}$.

1,4-Bis(4-bromobenzyl)oxy)anthracene-9,10-dione (4). Yield (%): 76 (1.82 g), mp 226-227 °C. Anal Calcd (%) for $C_{28}H_{18}Br_2O_4$ (578.3 g/mol): C 58.16, H 3.14. Found (%): C 58.10, H 3.07. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.14-8.11 (*m*, 2H, Ar-H), 7.67-7.65 (*m*, 2H, Ar-H), 7.48 (*d*, 4H, Ar-H, *J* 8 Hz), 7.41 (*d*, 4H, Ar-H, J = 8 Hz), 7.19 (*s*, 2H, Ar-H), 5.12 (*s*, 4H, CH_2) ppm. ^{13}C-NMR ($CDCl_3$, 100 MHz): 183.1 (C=O), 153.2, 135.5, 134.2, 133.5, 131.8, 128.8, 126.6, 124.2, 122.5, 121.9 (Ar-C), 71.4 (CH_2) ppm. IR: 1666 $\nu_{(C=O)}$, 1564 $\nu_{(quinone)}$, 1244 cm^{-1} $\nu_{(arom.\ CH)}$.

1,4-Bis(4-phenylbenzyl)oxy)anthracene-9,10-dione (5). A mixture of 1,4-Bis(4-bromobenzyl)oxy)anthracene-9,10-dione (0.58 g, 1 mmol), benzeneboronic acid (0.25 g, 2 mmol), K_2CO_3 (0.28 g, 2 mmol), $Pd(OAc)_2$ (% 2 mmol), PPh_3 (% 4 mmol), and DMF:H_2O (5:1 mL) was heated at 120 °C for 6h. After the reaction was terminated, the mixture was extracted with chloroform and water. A red-brown colored product was obtained. Yield (%): 51 (0.29 g), mp 153-154 °C. Anal Calcd (%) for $C_{40}H_{28}O_4$ (572.7 g/mol): C 83.90, H 4.93. Found (%): C: 83.69, H: 4.76. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.26-8.23 (*m*, 2H, Ar-H), 7.77-7.74 (*m*, 2H, Ar-H), 7.68-7.62 (*m*, 12H, Ar-H), 7.47 (*t*, 4H, Ar-H, *J* 8Hz), 7.38-7.28 (*m*, 4H, Ar-H), 5.33 (*s*, 4H, CH_2) ppm. ^{13}C-NMR ($CDCl_3$, 100 MHz): 183.4 (C=O),153.4, 140.9, 140.8, 135.6, 134.3, 133.3, 128.8, 127.6, 127.5, 127.4, 127.1, 126.5, 124.1, 122.7 (Ar-C), 71.9 (CH_2) ppm. IR: 1668 $\nu_{(C=O)}$, 1564 $\nu_{(quinone)}$, 1242 cm^{-1} $\nu_{(arom.\ CH)}$.

1,4-Bis(3-cyanopropyl)oxy)anthracene-9,10-dione (6). Yield (%): 64 (1.00 g), mp 227-228 °C. Anal Calcd (%) for $C_{22}H_{18}N_2O_4$ (374.4 g/mol): C 70.58, H 4.85, N 7.48. Found (%): C 70.30, H 4.68, N 7.35. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.18-8.12 (*m*, 2H, Ar-H), 7.77-7.70 (*m*, 2H, Ar-H), 7.30 (*t*, 2H, Ar-H, *J* 8 Hz), 4.22 (*t*, 4H, O-CH_2, *J* 8 Hz), 2.92 (*t*, 4H, CH_2-CN, *J* 8 Hz), 2.29 (*quint*, 4H, CH_2, *J* 8 Hz) ppm. ^{13}C-NMR ($CDCl_3$, 100 MHz): 182.9 (C=O), 153.4, 134.0, 133.5, 126.4, 123.5, 122.0 (Ar-C), 119.5 (CN), 67.5 (O-CH_2), 25.6 (*C*H_2CN), 14.2 (CH_2) ppm. IR: 2241 $\nu_{(CN)}$, 1664 $\nu_{(C=O)}$, 1561 $\nu_{(quinone)}$, 1243 cm^{-1} $\nu_{(arom.\ CH)}$.

1,8-Bis(4-methoxybenzyl)oxy)anthracene-9,10-dione (7). Yield (%): 73 (1.45 g), mp 205-206 °C. Anal Calcd (%) for $C_{30}H_{24}O_6$ (480.5 g/mol): C 74.99, H 5.03. Found (%): C 74.82, H 5.00. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.86 (d, 2H, Ar-H, *J* 8H), 7.60 (t, 2H, Ar-H, *J* 8H), 7.55 (d, 2H, Ar-H, *J* 8H), 7.34 (d, 2H, Ar-H, *J* 8H), 6.93 (d, 2H, Ar-H, *J* 8H), 5.27 (s, 4H, CH_2), 3.84 (s, 6H, OCH_3) ppm. ^{13}C-NMR ($CDCl_3$, 100 MHz): 182.4 (C=O), 159.3, 158.3, 134.9, 133.6, 128.6, 128.5, 125.1, 120.6, 119.4, 114.0 (Ar-C), 71.1 (CH_2), 55.3 (OCH_3) ppm. IR: 1666 $\nu_{(C=O)}$, 1584 $\nu_{(quinone)}$, 1235 cm^{-1} $\nu_{(arom.\ CH)}$.

1,8-Bis(4-cyanobenzyl)oxy)anthracene-9,10-dione (8). Yield (%): 71 (1.40 g), mp 272-273 °C. Anal Calcd (%) for $C_{30}H_{18}N_2O_4$ (470.5 g/mol): C 76.59, H 3.86, N 5.95. Found (%): C 76.33, H 3.74, N 5.83. ^{1}H-NMR (DMSO-d_6, 400 MHz): 7.91-7.83 (*m*, 8H, Ar-H), 7.79-7.74 (*m*, 4H, Ar-H), 7.61 (*d*, 2H, Ar-H, *J* 8 Hz), 5.44 (*s*, 4H, CH_2) ppm. ^{13}C-NMR (DMSO-d_6, 100 MHz): 183.5 (C=O), 157.8, 143.2, 134.9, 134.7, 132.8, 127.9, 124.3, 120.8, 119.4, 110.8 (Ar-C), 119.2 (CN), 69.8 (CH_2) ppm. IR: 2228 $\nu_{(CN)}$, 1668 $\nu_{(C=O)}$, 1586 $\nu_{(quinone)}$, 1297 cm^{-1} $\nu_{(arom.\ CH)}$.

1,8-Bis(4-bromobenzyl)oxy)anthracene-9,10-dione (9). Yield (%): 75 (1.80 g), mp 233-234 °C. Anal Calcd (%) for $C_{28}H_{18}Br_2O_4$ (578.3 g/mol): C 58.16, H 3.14. Found (%): C 57.95, H 3.08. ^{1}H-NMR (DMSO-d_6, 400 MHz): 7.81-7.73 (*m*, 4H, Ar-H), 7.63-7.60 (*m*, 10H, Ar-H), 5.30 (*s*, 4H, CH_2) ppm. ^{13}C-NMR (DMSO-d_6, 100 MHz): 183.5 (C=O), 157.9, 136.8, 134.8, 134.7, 131.7, 129.6, 124.5, 121.2, 120.9, 119.2 (Ar-C), 69.9 (CH_2) ppm. IR: 1666 $\nu_{(C=O)}$, 1564 $\nu_{(quinone)}$, 1244 cm^{-1} $\nu_{(arom.\ CH)}$.

1,8-Bis(4-chlorobenzyl)oxy)anthracene-9,10-dione (10). Yield (%): 79 (1.61 g), mp 231-232 °C. Anal Calcd (%) for $C_{28}H_{18}Cl_2O_4$ (489.4 g/mol): C 68.73, H 3.71. Found (%): C 68.55, H 3.60. ^{1}H-NMR (DMSO-d_6, 400 MHz): 7.81-7.73 (m, 4H, Ar-H), 7.67-7.61 (m, 6H, Ar-H), 7.46 (d, 4H, Ar-H, *J* 8 Hz), 5.32 (s, 4H, CH_2). ^{13}C-NMR (DMSO-d_6, 100 MHz): 181.9 (C=O), 157.9, 136.5, 134.8, 132.7, 129.3, 128.8, 124.3, 120.9, 119.2, 115.6 (Ar-C), 69.9 (CH_2) ppm. IR: 1668 $\nu_{(C=O)}$, 1584 $\nu_{(quinone)}$, 1281 cm^{-1} $\nu_{(arom.\ CH)}$.

2,2'-(((9,10-Dioxo-9,10-dihydroanthracene-1,4-diyl)bis(oxy))bis(ethane-2,1-diyl))bis(isoindoline-1,3-dione) (11). Yield (%): 73 (1.78 g), mp 265-266 °C. Anal Calcd (%) for $C_{34}H_{22}N_2O_8$ (586.6 g/mol): C 69.62, H 3.78, N: 4.78. Found (%): C 69.52, H 3.70, N 4.62. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.84-7.82 (m, 4H, Ar-H), 7.75 (d, 2H, Ar-H, *J* 8 Hz), 7.68-7.66 (m, 4H, Ar-H), 7.50 (t, 2H, Ar-H, *J* 8 Hz), 7.26-7.24 (m, 2H, Ar-H), 4.32 (t, 4H, CH_2, *J* 8 Hz), 4.15 (t, 4H, CH_2, *J* 8 Hz) ppm. ^{13}C-NMR ($CDCl_3$, 100 MHz): 183.7 ($C=O_{anthraquinone}$), 168.2 ($C=O_{phthalimide}$), 157.9, 134.8, 134.1, 134.0, 133.6, 132.2, 125.3, 123.4, 123.3, 120.8, 119.9 (Ar-C), 66.4 (CH_2), 37.0 (CH_2) ppm. IR: 1705 $\nu_{(C=O\ imide)}$, 1670 $\nu_{(C=O\ anthraquinone)}$, 1583 $\nu_{(quinone)}$, 1237 cm^{-1} $\nu_{(arom.\ CH)}$.

In vitro inhibition of xanthine oxidase (XO) activity

The enzyme activity were assayed by measuring the uric acid formation at 294 nm at 37 °C. The *in vitro* XO inhibitory assay method was reported by Sweeney A.P. et al.[49] For calculation IC_{50} value of XO inhibition different concentrations of anthraquinone derivatives were added to the reaction mixture. In brief, the enzyme assay protocol contains phosphate buffer (50 mM, pH = 7.4), XO (0.2 U), and xanthine (1 mM). The enzyme was pre-incubated for (10 min) with tested compounds, then the reaction was started by adding xanthine to the reaction mixture. All the experiments were performed in triplicates, and values were expressed as means of three experiments. Allopurinol was used as a positive control. The IC_{50} values were determined for based compounds were measured as percent inhibition of XO was studied in terms of decrease in uric acid formation as compared to the product formation in the absence of inhibitor.

The percent inhibition (IC_{50} of XO) activities of compounds were calculated related to this formula. Inhibition (%) = (A-B)/A×100; A = the absorbance at 294 nm without the test compound, B = the absorbance at 294 nm with the test compound.

Acknowledgements

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Supplementary Material

^{1}H, ^{13}C NMR, and FT-IR spectra of compounds are accessible via the "Supplementary Material" section of this article's webpage.

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