Synthesis and pharmacological evaluation of aniline derivatives as a potent analgesic and antioxidant agent

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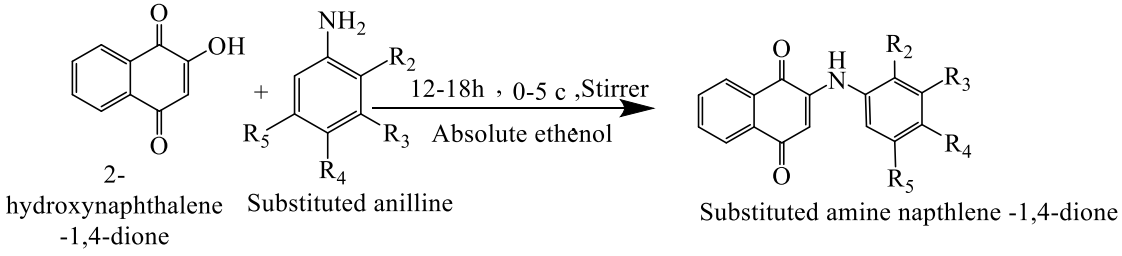
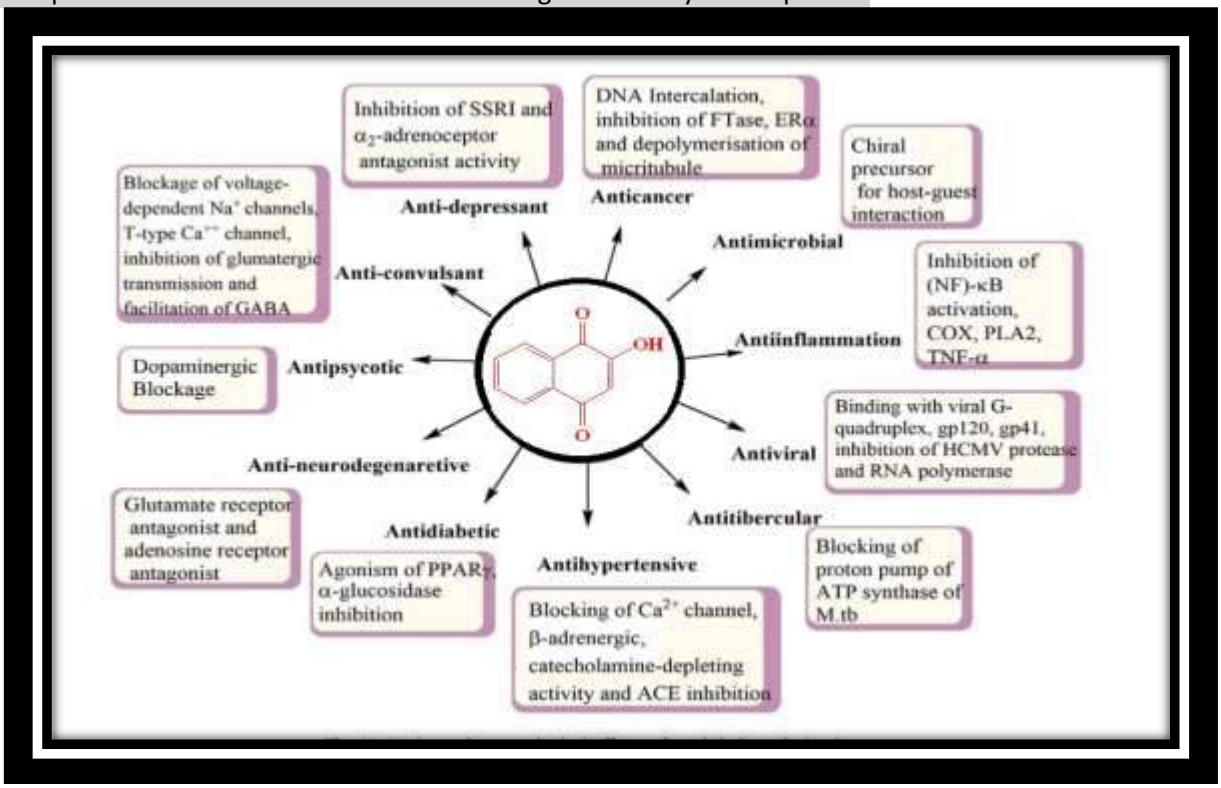
Graphical abstract:-



1, 4- naphthoquinone (Lawsone) is widely distributed in nature and has been used since ancient times in traditional medicine. Lawsone has been used as a dye, and both its natural form and synthetic derivatives exhibit antifungal, antibacterial, antitumor, Antimalarial, molluscicidal, antipsychotic, anticonvulsant, antidepressants, antihypertensive, and Antidiabetic, anti-neurodegenerative and

antioxidant activity. 1, 4- naphthoquinone (Lawsone) was isolated from the leaves of Lawsonia inermis by using the pH gradient (agitation) & maceration method. A convenient synthesis of 2-substituted amino naphthalene-1, 4-dione (3a-o) has been achieved by reaction of isolated 1, 4- naphthoquinone with substituted aniline in the presence of ethanol and DPPH model is used for the evaluation of the antioxidant activities and in vivo analgesic activity using albino mice morphine-induced hot plate method. The structure of the ultimate analogs has been inveterate on the basis of elemental analysis, IR, ^{1}H NMR, mass spectra and elemental analysis. All the standards of elemental investigation, IR, ^{1}H NMR, and mass spectra were initiate to be prominent. The results indicate that synthesized compound 3d having IC50 75.39 ± 4.12 mg/ml showed potent antioxidant activity comparable to standard ascorbic acid (IC50 45.54 ± 3.06 mg/ml). 3c, 3m, and 3o showed potent analgesic activity comparable to standard nimesulide (100% at 50 mg/kg b.w). This reading suggests that leaves of Lawsonia inermis have bioactive compounds for innovative antioxidant & analgesic remedy development.



KEYWORDS: Lawsonia inermis, Substituted Aniline, Naphthoquinone, DPPH assay, Eddy hot plate.

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Introduction

Naphthoquinone derivatives have pulled in proceeding with enthusiasm throughout the years due to the utilization of its ring framework as a vital center constitution in numerous medicine substances and are reported to cover up a broad variety of pharmacological implementation [1-2]. Due to their varied pharmacological qualities, such as their antioxidant activity, plant-derived natural products such as phenolic compounds (flavonoids), steroids, terpenoids, saponins, volatile oils, glycosides, etc. have drawn a lot of attention in recent years. Furthermore, 1, 4 naphthoquinones in particular are abundantly dispersed. Naphthoquinones are phenolic compounds that are believed to have a variety of pharmacological activities, including antibacterial, antifungal, antiviral, anti-inflammatory, antipyretic, and anticancer action. [4, 24]. Humans are protected against

infection and degenerative diseases by antioxidants, which are crucial in blocking and scavenging free radicals [5]. Although experiments and clinical trials involving entire animals are imperative in plant product assaying however the significance of in vitro assaying is picking up prevalence because of money-related, moral, and time requirements. The aptitude to quickly recognize active compounds in the intricate assortment of plant product extract, lead optimization as well as lead characterization are significant factors in plant product assaying. Natural plant-based antioxidants have been extensively studied, and their value in food, health, and preventative medicine is well known [6-10]. The current study reports on a comparison of the in-vitro antioxidant activity of various naphthoquinone derivatives, which may aid in the discovery of new antioxidant compounds (s). Because of the above mention and as a part of our continuous efforts towards the development of more potent antioxidant & analgesic agents [11]. It was thought of interest to combine the above-mentioned boilable rings in a molecular framework to investigate the additive effect of these rings on antioxidant & analgesic activities.

1. Chemistry

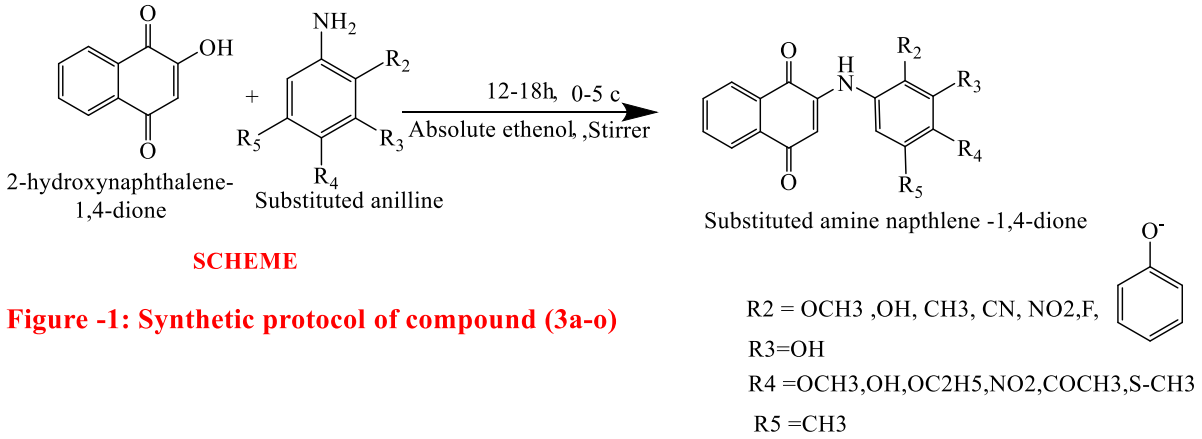
Naphthoquinone (1) (300 mg, 1.89 mmol) in 20 mL absolute ethanol was stirred at 5-10 ºC until the solid completely dissolved. To this naphthoquinone, the solution was added dropwise a solution of the corresponding amine (2) (0.95 mmol) in 10 mL of absolute ethanol at 5-10 ºC. The reaction mixture was allowed to reach room temperature and was stirred for another 12–18 h. The completion of the reaction was monitored by TLC. The solid product thus obtained was filtered, washed with water, and Recrystallize from acetone to give compounds (3a-o) [12-13]. Elemental analysis, yield, and melting points were given in Table 1. This protocol is very significant because of its specific generation of crystalline products. The synthesized compounds were characterized by IR, ^{1}H NMR, ESI-MS, and elemental analysis.

2. Pharmacological evaluation

All the compounds prepared here were assayed for their pharmacological actions for instance in-vivo palliative and in vitro antioxidant behavior. The physiological reactions of animals to heat and chemical stimuli were measured during these activities. For palliative activity in mice [20-21] at 50 mg/kg body weight (b.w) have performed. The % of fortification has deliberate compared to standard drug nimesulide. The entire compounds showed persuasive activity compared with Nimesulide. For antioxidants activity, the DPPH method is used. The % of fortification has calculated compared to standard drug ascorbic acid. The entire compounds showed persuasive activity compared with ascorbic acid.

4. Results and discussion

4.1. Chemistry



SCHEME

Figure -1: Synthetic protocol of compound (3a-o)

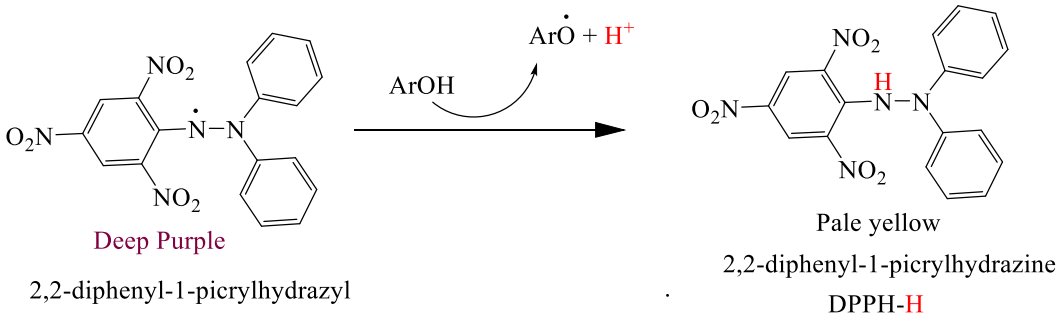
1, 4- naphthoquinone derivatives with various substituted aromatic amines by stirring in ethanol for 12-18 h yielded substituted (substituted aniline) naphthalene-1, 4-dione derivatives (a-o) according to the literature method [22]. The maintenance of minimum temperature (5 ºC) is very important as it led to crystalline products. The corresponding yields (57–89%) were obtained in good agreement. The postulated structures of newly synthesized targeted naphthalene-1,4-dione derivatives are under the elemental analysis, IR, ^{1}H NMR, and ESI-MS. IR spectra of all the compounds showed stretching bands at 3402–3590 cm^{-1} of aromatic –NH respectively. Further (C-N) stretching bands appeared at 1552–1585 cm^{-1} respectively. The ^{1} H NMR spectral broad singlet signals were observed at d 2.12–5.2 NH protons of aliphatic and NH protons of naphthalene-1,4-dione are merged with aromatic and are D2O exchangeable. ESI-MS spectra showed the corresponding molecular ion peaks for all the compounds. All the spectral results of 2-(substituted aniline) naphthalene-1,4-dione derivatives (a-o) are tabulated in Table 2.

4.2. Analgesic activity

The analgesic activity of the synthesized compounds was assessed by the morphine-induced hot plate method. Almost all the compounds have shown very potent analgesic activity when compared with standard nimesulide drugs. Among the tested compounds 2-(4-hydroxyphenylamino)naphthalene-1, 4-dione (3c), 2-((2,4-dinitrophenyl)amino)naphthalene-1,4-dione (3m), 2-((2-fluorophenyl)amino)naphthalene-1,4-dione (3o) showed pronounced analgesic activity (89%, 100 mg/kg b.w). The remaining compounds 3a, 3b, 3d, 3e, 3f, 3g, 3h, 3i, 3j, 3k, 3l, and 3n have also shown good activity because methoxy, hydroxy, methyl, nitro, methylthio, phenoxy, and ethoxy substituted anilines. Within the same ring system i.e., substituted anilines, it was noticed that the introduction of 2, 4-dihydroxy, and methyl groups in the naphthoquinone ring and nitro group in the aniline at different positions enhanced the analgesic activity. Several studies suggested the analgesic effect of the derivative and formulating with several nanogels to obtain maximum activity [23].

4.3 Antioxidants activity

DPPH assay was discovered by **Goldschmidt** and **Renn** in the 1920s. It is a stable free radical with purple color which turns yellow when scavenged. DPPH is used for measuring the total antioxidant capacity (TAC). Antioxidant capacity is the overall ability of organisms or food to catch free radicals and prevents their harmful effects.



Deep Purple

2,2-diphenyl-1-picrylhydrazyl

Pale yellow

2,2-diphenyl-1-picrylhydrazine

DPPH-H

DPPH (1, 1-diphenyl-2-picrylhydrazyl) technique is the most excellent, straightforward, and most frequently used technique for evaluating prelude free radical-scavenging activity [14-15]. In chemical analysis, the stable free radical DPPH is typically used to identify the action of radical scavengers. It has an unusual electron in its structure. Labile hydrogen is known to be abstracted by DPPH. [16-17]

Table -1

Physical data for synthesized compounds & isolated yield

Compounds	Molecular formula	Yield (%)	MP(°C)	C (%)		N (%)		H (%)		O%	
				Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found
A	$C_{17}H_{13}NO_3$	57%	196	73.15	73.11	5.07	5.02	4.76	4.69	17.23	17.19
B	$C_{17}H_{13}NO_3$	68%	ºC	5	73.11	5.07	5.02	4.76	4.69	3	17.19
C	$C_{16}H_{11}NO_3$	66%	190	73.15	72.45	5.34	5.28	4.23	4.18	17.23	18.09
D	$C_{16}H_{11}NO_4$	73%	ºC		68.33	5.08	4.98	3.98	3.94		22.75
E	$C_{17}H_{13}NO_2$	87%	192	72.51	77.55	5.41	5.32	5.07	4.98	18.16	12.15
F	$C_{18}H_{15}NO_2$	73%	ºC		77.96	5.08	5.05	5.56	5.49		11.54
G	$C_{18}H_{15}NO_3$	82%	263	68.49	73.71	4.83	4.78	5.22	5.15	22.84	16.36
H	$C_{16}H_{11}NO_3$	74%	ºC		72.45	5.34	5.28	4.26	4.18		18.09
I	$C_{16}H_{10}N_2O_4$	69%	227	77.61	65.31	9.57	9.52	3.49	3.43	12.22	21.75
J		63%	ºC		69.13	4.79	4.74	4.56	4.44		10.83
K	$C_{17}H_{13}NO_2S$	67%	202	78.02	74.44	10.33	10.21	3.73	3.68	11.67	11.67
L		68%	ºC		74.22		4.81	4.56	4.50		16.48
M	$C_{17}H_{10}N_2O_2$	76%	210	73.76	56.65	4.89	12.39	2.75	2.67	16.45	28.29
N		69%	ºC		77.41	12.46	4.10	4.49	4.43		14.06
O	$C_{18}H_{13}NO_3$	67%	198	72.51	71.91		5.24	3.84	3.77	18.19	11.97
	$C_{16}H_9N_3O_6$		ºC			4.16					
	$C_{22}H_{15}NO_3$		210	65.36		5.32				21.83	
	$C_{16}H_{10}FNO_2$		ºC								
			220	69.17						10.89	
			ºC								
			224	74.52						11.76	
			ºC								
			218	74.32						16.57	
			ºC								
			224	74.73						28.38	
			ºC								
			228	77.46						14.17	
			ºC								
			194	71.97						12.09	
			ºC								

Conclusion

In conclusion, we have described a simple and efficient protocol for the preparation of 2-(2-substituted aryl amino) naphthalene-1, 4-dione derivatives with excellent yields. All the synthesized compounds have been screened for their in-vivo analgesics and vitro antioxidant activities. In newly synthesized compounds, it is clear that the highest analgesic activity in compounds 3c, 3m, 3o, and antioxidants activity in compound 3d was observed. Apart from compounds 3c, 3m, and 3o, the remaining compounds have shown good analgesic activity almost equal to standard nimesulide drugs. compounds 3a,3b,3d,3e,3f,3g,3h,3i,3j,3k,3l and 3n have shown moderate antioxidant activity .the preliminary in vivo studies for analgesics as well as in vitro studies for antioxidants the compounds evidenced that the chloro group, methoxy, phenyl as well as the nitro group in

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the meta position in the aniline ring enhance the analgesics as well as antioxidants activities, They could work as fresh models in the creation of strong treatments; as a result, it can be said that these substances exhibit pharmacological activity. This has had a positive effect on chemists and biochemists who are now looking into drug design further in order to find analgesics and antioxidants with halo, nitro, methyl, and phenyl functional groups.

Experimental protocols

Collection of Plant Material

The whole plants of *Lawsonia inermis* were collected from several habitats in the Amethi & Shravasti district region of Uttar Pradesh, India. *Lawsonia inermis* was identified and authenticated by Dr. Sunita Garg former chief scientist, and head, at RHMD, CSIR-NIScPR. A voucher specimen no (NIScPR/RHMD/Consult/2021/3982-83) was deposited in the raw material herbarium and museum, Delhi (MHRD). And the leaves were washed in running water and air-dried.

Chemicals

These were the purchases. Organic solvents: petroleum ether, DMSO, and diethyl ether, from Central Drug House (P) Ltd.; acetone, chloroform, n-hexane, ethyl acetate, and ethanol from Qualigens® Fine Chemicals (Mumbai) (New Delhi), and ethanol from Changshu Hongsheng fine chemical co., ltd. Changshu city ,Jiangsu Province. (all analytical grades). 1,1-Diphenyl-2-picrylhydrazyl radical from Sigma-Aldrich (New Delhi). Other reagents used were of analytical grade and obtained from different commercial sources.

Material and method

The melting points of the products were determined by open capillaries on a Thiele tube Fisher john's apparatus and are found to be 196 °C. The IR spectra were recorded on a Shimadzu Ftir-8400s Spectrophotometer, using KBr pellets. ^{1}H NMR spectra were recorded on a Bruker Avance 400/AvIII HD300 (FT NMR) spectrometer in CDCl3 using TMS as an internal standard with a ^{1}H resonant frequency of 300 MHz. The Mass spectra were recorded on a Waters Alliance e2695/HPLCTQD Mass spectrometer (ESI-MS) The EuroVector analyzer was used to do the elemental analysis. All of the compounds produced C, H, O, and N analyses that were within 5% of the predicted values. TLC was used to describe the homogeneity of the compounds on aluminium silica gel 60 F254 (Merck), which was detected by UV light (254 nm) and iodine vapors. All reagents were analytical graded or chemically pure.

Isolation and Purification of Lawsone

Shade dried and 200 g powered henna are extracted by agitation for 2 h with 20% sodium bicarbonate solution (800 ml). The extract was filtered, marc is re-extracted with 400 ml of the same solution for 1hr, filtered and the alkaline extract was pooled together. The extract was acidified with dil. sulphuric acid and crude product obtained on standing was re-extracted with a sufficient quantity of ammonium hydroxide and again acidified with dil. hydrochloric acid. The product is finally extracted with two successive quantities of benzene (160 ml) and filtered [12]. The filtrate was distilled to yield a yellow-brown color crystal of lawsone and the purity was checked by TLC. The red spot obtained had an Rf value of 0.07 and its melting point was obtained as 196 ^{o}C.

General procedure for the synthesis of 2-(2-substituted) naphthalene-1, 4-Dione (3a-o)

Naphthoquinone (1) (300 mg, 1.89 mmol) in 20 mL absolute ethanol has stirred at 5-10 ºC in anticipation of the solid completely solvated. To this naphthoquinone, Dropwise, a solution of the appropriate amine (2) (0.95 mmol) has been added to the reaction mixture in 10 mL of absolute ethanol at 5-10 ºC. The homogenate was brought to room temperature and agitated for a further 12 to 18 hours. The completion of the reaction was monitored by TLC. The solid product thus obtained was filtered, washed with water, and recrystallized from acetone to give compounds (3a-o) [13].

2.5.2. 2-(2-methoxyphenyl-amino) naphthalene-1, 4-dione (3a) :-Yield 57%;Red solid; mp 196 ºC; eluent-n-hexane/ethyl acetate

70:30 v/v, Rf = 0.67; IR (KBr, υ, cm-1): 3100 (Ar C-H), 1685, 1676 (C=O, α,β unsaturated), 1285 (C-N), 3403 (N-H), 1570 (C=C), 1204 (C-O-C asym.), 1033 (C-O-C sym); ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 3.74 (s, 3H, OCH3), 4.17 (s, 1H, H-N, D2O exchangeable), 6.31-7.02 (m, 4H, Ar-H), 7.92-8.15 (d, 2H, Ar-H), 8.19-8.22 (dd, 2H, Ar-H), 8.43 (s, 1H, Ar-H); EIMS; Found, (m/z): 280.9290. $[M+H]^+$. C17H13NO3. Calculated, 279.8954.

2.5.3. 2-(4-methoxyaniline) naphthalene-1, 4-dione (3b):- Yield 68%; Red solid; mp 190 ºC; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.71; IR (KBr, υ, cm-1): 3055 (Ar C-H), 1655, 1678 (C=O, α,β unsaturated), 1277 (C-N), 3409 (N-H), 1590 (C=C), 1230 (C-O-C asym.), 1028 (C-O-C sym); 1 H NMR (300 MHz, DMSO-d6) δ (ppm): 3.72 (s, 3H, OCH3), 4.05 (s, 1H, NH, D2O exchangeable), 6.88-6.92 (m, 4H, ArH), 7.66-7.90 (d, 2H, Ar-H), 8.06-8.11 (dd, 2H, Ar-H), 8.23 (s, 1H, ArH); EIMS; Found, (m/z): 280.9290 . $[M+H]^+$. C17H13NO3. Calculated, 279.8954.

2.5.4. 2-(4-hydroxyphenylamino) naphthalene-1, 4-dione (3c) :-Yield 66 %; Brown solid; mp 192 ºC; eluent-n-hexane/ethyl acetate 70:30 v/ v, Rf = 0.63; IR (KBr, υ, cm-1): 3107 (Ar C-H), 1645, 1654 (C=O, α, β unsaturated), 1256 (C-N), 3402 (N-H), 1509 (C=C), 1102 (C-O), 3599 (O-H); 1 H NMR (300 MHz, DMSO-d6) δ (ppm): 4.73 (s, 1H, NH, D2O exchangeable), 5.02 (s, 1H, OH, D2O exchangeable), 6.02-6.95 (m, 4H, Ar-H), 7.12-7.89 (d, 2H, Ar-H), 7.99-8.43 (dd, 2H, Ar-H); EIMS Found, m/z: 266.7725 $[M+H]^+$. C16H11NO3. Calculated, m/z: 265.7389.

2.5.5. 2-(2, 4-dihydoxyphenylamino) naphthalene-1, 4-dione (3d):- Yield 73 %; Brown solid; mp 263 ºC ; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.68; IR (KBr, υ, cm-1): 3102 (Ar C-H), 1633, 1687 (C=O, α,β unsaturated),), 1282 (C-N), 3412 (N-H), 1554 (C=C), 1209 (C-O), 3599 (O-H); 1 H NMR (300 MHz, DMSO-d6) δ(ppm): 4.36 (s, 1H, NH, D2O exchangeable), 5.02 (s, 2H, OH, D2O exchangeable), 6.85- 6.97 (m, 6H, Ar-H), 7.88-7.92 (d, 2H, Ar-H); EIMS Found, m/z: 282.7216 $[M+H]^+$. C16H11NO4. Calculated, m/z: 281.6881.

2.5.6. 2-(-p-tolylamino)naphthalene-1, 4-dione (3e):-Yield 87 %; Yellow crystal; mp 227 ºC; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.77; IR (KBr, υ, cm-1): 3035 (Ar C-H), 1635, 1640 (C=O, α,β unsaturated), 1272 (C-N), 3490 (N-H), 1535 (C=C), 1289 (C-O), 3578 (O-H); 1 H NMR (300 MHz, DMSO-d6) δ (ppm): 2.32 (s, 3H, CH3), 4.28 (s, 1H, NH, D2O exchangeable), 6.22-6.91 (m, 4H, Ar-H), 7.19-7.27 (d, 2H, Ar-H), 7.60-7.74 (dd, 2H, Ar-H), 7.96 (s, 1H, Ar-H); EIMS Found, m/z: 264.9798 $[M+H]^+$. C17H13NO2. Calculated, m/z: 263.9463.

2.5.7. 2-((3, 4-dimethyl phenyl) amino) naphthalene-1,4-dione (3f):-Yield 73 %; Yellow crystal; mp 202 ºC; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.73; IR (KBr, υ, cm-1): 3135 (Ar C-H), 16355, 1660 (C=O, α,β unsaturated), 1292 (C-N), 3570 (N-H), 1635 (C=C); ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 2.22 (s, 3H, CH3), 4.18 (s, 1H, NH, D2O exchangeable), 6.27-7.21 (m, 4H, Ar-H), 7.49-7.87 (d, 2H, Ar-H), 7.50-7.64 (dd, 2H, Ar-H), 7.76 (s, 1H, Ar-H); EIMS, Found, m/z: 278.1136 $[M+H]^+$. C18H15NO2. Calculated, m/z: 277.1102.

2.5.8. 2-((4-ethoxy phenyl) amino) naphthalene-1, 4-dione (3g):-Yield 82 %; Yellow crystal; mp 210 ºC; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.76; IR (KBr, υ, cm-1): 3070 (Ar C-H), 1645, 1650 (C=O, α,β unsaturated), 1278 (C-N), 3495 (N-H), 1545 (C=C), 1299 (C-O), 3576 (O-H); 1 H NMR (300 MHz, DMSO-d6) δ (ppm): 2.33 (s, 3H, CH_3), 4.29 (s, 1H, NH, D2O exchangeable), 6.23-6.92 (m, 4H, Ar-H), 7.29-7.37 (d, 2H, Ar-H), 7.65-7.79 (dd, 2H, Ar-H), 7.86 (s, 1H, Ar-H); EIMS Found, m/z: 294.1085 $[M+H]^+$. C18H15NO3. Calculated, m/z: 293.1051.

2.5.9. 2-((4-hydroxyphenyl)amino)naphthalene-1,4-dione (3h):-Yield 74 %; Yellow crystal; mp 198 ºC; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.71; IR (KBr, υ, cm-1): 3028 (Ar C-H), 1632, 1637 (C=O, α,β unsaturated), 1253 (C-N), 3498 (N-H), 1525 (C=C), 3593 (O-H); ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 2.33 (s, 3H, CH3), 4.38 (s, 1H, NH, D2O exchangeable), 6.16-6.85

(m, 4H, Ar-H), 7.16-7.27 (d, 2H, Ar-H), 7.60-7.69 (dd, 2H, Ar-H), 7.94 (s, 1H, Ar-H); EIMS Found, m/z: 266.7725 $[M+H]^+$. C16H11NO3. Calculated, m/z: 265.7389.

2.5.10. 2-((4-nitrophenyl) amino) naphthalene-1, 4-dione (3i):-Yield 69 %; Yellow crystal; mp 210 ºC; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.67; IR (KBr, υ, cm-1): 3025 (Ar C-H), 1615, 1620 (C=O, α,β unsaturated), 1252 (C-N), 3490 (N-H), 1525 (C=C); ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 2.12 (s, 3H, CH3), 4.18 (s, 1H, NH, D2O exchangeable), 6.13-6.90 (m, 4H, Ar-H), 7.17-7.25 (d, 2H, Ar-H), 7.50-7.64 (dd, 2H, Ar-H), 7.94 (s, 1H, Ar-H); EIMS Found, m/z: 295.6741 $[M+H]^+$. C16H10N2O4. Calculated, m/z: 294.6406.

2.5.11. 2-((4-(methylthio) phenyl) amino) naphthalene-1, 4-dione (3j):-Yield 63 %; Yellow crystal; mp 220 ºC; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.76; IR (KBr, υ, cm-1): 3035 (Ar C-H), 1635, 1720 (C=O, α,β unsaturated), 1272 (C-N), 3590 (N-H), 1635 (C=C); ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 2.34 (s, 3H, CH3), 4.26 (s, 1H, NH, D2O exchangeable), 6.18-6.88 (m, 4H, Ar-H), 7.29-7.37 (d, 2H, Ar-H), 7.50-7.64 (dd, 2H, Ar-H), 7.66 (s, 1H, Ar-H); EIMS Found, m/z: 296.7005 $[M+H]^+$. C17H13NO2S. Calculated, m/z: 295.6670.

2.5.12. 2-((1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)benzonitrile (3k):-Yield 67 %; Yellow crystal; mp 224 ºC; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.74; IR (KBr, υ, cm-1): 3095 (Ar C-H), 1735, 1740 (C=O, α,β unsaturated), 1282 (C-N), 3520 (N-H), 1645 (C=C); ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 2.34 (s, 3H, CH3), 4.32 (s, 1H, NH, D2 O exchangeable), 6.13-6.83 (m, 4H, Ar-H), 7.15-7.23 (d, 2H, Ar-H), 7.58-7.72 (dd, 2H, Ar-H), 7.98 (s, 1H, Ar-H); EIMS, Found, m/z: 275.7758 $[M+H]^+$. C17H10N2O2. Calculated, m/z: 274.7423.

2.5.13. 2-((4-acetyl phenyl)amino)naphthalene-1,4-dione (3l):-Yield 68 %; Yellow crystal; mp 218 ºC; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.73; IR (KBr, υ, cm-1): 3005 (Ar C-H), 1745, 1750 (C=O, α,β unsaturated), 1277 (C-N), 3480 (N-H), 1625 (C=C); ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 2.36 (s, 3H, CH3), 4.31 (s, 1H, NH, D2O exchangeable), 6.25-6.94 (m, 4H, Ar-H), 7.21-7.29 (d, 2H, Ar-H), 7.62-7.76 (dd, 2H, Ar-H), 7.94 (s, 1H, Ar-H); EIMS, Found, m/z: 292.9290 $[M+H]^+$. C18H13NO3. Calculated, m/z: 291.8954.

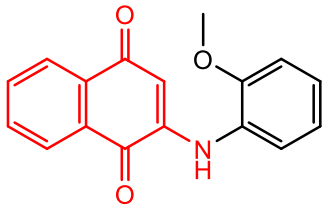
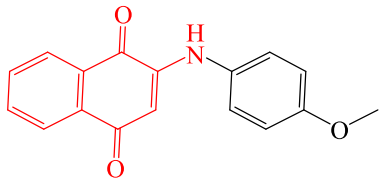
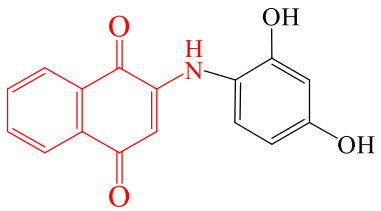
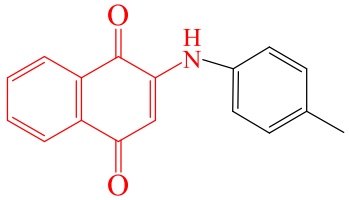
2.5.13. 2-((2,4-dinitrophenyl)amino)naphthalene-1,4-dione (3m):-Yield 76 %; Yellow crystal; mp 224 ºC; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.62; IR (KBr, υ, cm-1): 3015 (Ar C-H), 1625, 1630 (C=O, α,β unsaturated), 1267 (C-N), 3477 (N-H), 1655 (C=C), 1589 (N-O); ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 2.42 (s, 3H, CH3), 4.38 (s, 1H, NH, D2O exchangeable), 6.32-6.81 (m, 4H, Ar-H), 7.14-7.32 (d, 2H, Ar-H), 7.64-7.78 (dd, 2H, Ar-H), 7.93 (s, 1H, Ar-H); EIMS, Found, m/z: 340.5249 $[M+H]^+$. C16H9N3O6. Calculated, m/z: 339.4914.

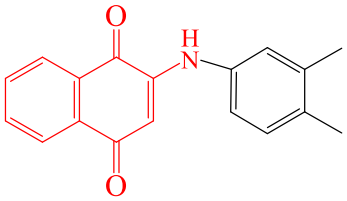
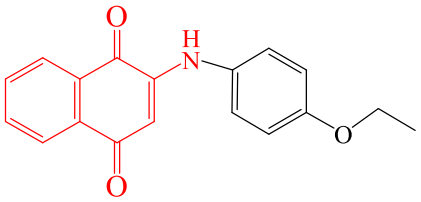
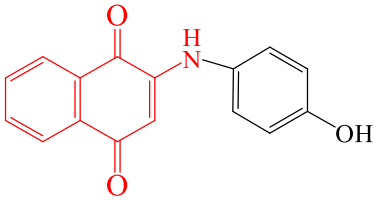
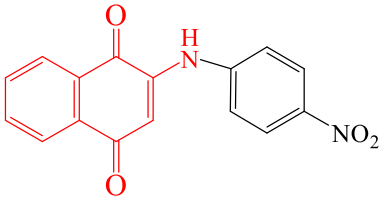
2.5.14. 2-((2-phenoxy phenyl)amino)naphthalene-1,4-dione (3n):-Yield 69 %; Yellow crystal; mp 228 ºC; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.71; IR (KBr, υ, cm-1): 3085 (Ar C-H), 1715, 1720 (C=O, α,β unsaturated), 1266 (C-N), 3580 (N-H), 1624 (C=C), 1301 (C-O); ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 2.32 (s, 3H, CH_3), 4.29 (s, 1H, NH, D2O exchangeable), 6.23-6.93 (m, 4H, Ar-H), 7.21-7.29 (d, 2H, Ar-H), 7.58-7.71 (dd, 2H, Ar-H), 7.89 (s, 1H, Ar-H); EIMS, Found, m/z: 342.1085 $[M+H]^+$. C22H15NO3. Calculated, m/z: 341.1051.

2.5.15. 2-((2-fluoro-phenyl) amino) naphthalene-1,4-dione (3o):-Yield 67 %; Yellow crystal; mp 194 ºC; eluent-n-hexane/ethyl acetate 70:30 v/v, Rf = 0.67; IR (KBr, υ, cm-1): 3135 (Ar C-H), 1605, 1610 (C=O, α,β unsaturated), 1282 (C-N), 3530 (N-H), 1635 (C=C); ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm): 2.31 (s, 3H, CH3), 4.27 (s, 1H, NH, D2O exchangeable), 6.27-6.83 (m, 4H, Ar-H), 7.17-7.22 (d, 2H, Ar-H), 7.54-7.71 (dd, 2H, Ar-H), 7.96 (s, 1H, Ar-H); EIMS Found, m/z: 268.7291 $[M+H]^+$. C16H10FNO2. Calculated, m/z: 267.6956.

Spectral analysis Table

-2

SI NO	Compound & structure	Infrared cm^{-1} (KBr pallets)	^{1} H Nuclear Magnetic Resonance Values in ppm	Mass At m/z (M+H)+
3a		3100 (Ar C-H), 1685, 1676 (C=O, α,β unsaturated), 1285 (C-N), 3403 (N-H), 1570 (C=C), 1204 (C-O-C asym.), 1033 (C-O-C sym)	3.74 (s, 3H, OCH_3), 4.17 (s, 1H, H-N, D2O exchangeable), 6.31-7.02 (m, 4H, Ar-H), 7.92-8.15 (d, 2H, Ar-H), 8.19-8.22 (dd, 2H, Ar-H), 8.43 (s, 1H, Ar-H)	279.8954
3b		3055 (Ar C-H), 1655, 1678 (C=O, α,β unsaturated), 1277 (C-N), 3409 (N-H), 1590 (C=C), 1230 (C-O-C asym.), 1028 (C-O-C sym)	3.72 (s, 3H, OCH3), 4.05 (s, 1H, NH, D2O exchangeable), 6.88-6.92 (m, 4H, ArH), 7.66-7.90 (d, 2H, Ar-H), 8.06-8.11 (dd, 2H, Ar-H), 8.23 (s, 1H, ArH)	279.8954
3c		3107 (Ar C-H), 1645, 1654 (C=O, α, β unsaturated), 1256 (C-N), 3402 (N-H), 1509 (C=C), 1102 (C-O), 3599 (O-H)	4.73 (s, 1H, NH, D2O exchangeable), 5.02 (s, 1H, OH, D2O exchangeable), 6.02-6.95 (m, 4H, Ar-H), 7.12-7.89 (d, 2H, Ar-H), 7.99-8.43 (dd, 2H, Ar-H)	265.7389
3d		3102 (Ar C-H), 1633, 1687 (C=O, α,β unsaturated),), 1282 (C-N), 3412 (N-H), 1554 (C=C), 1209 (C-O), 3599 (O-H)	4.36 (s, 1H, NH, D2O exchangeable), 5.02 (s, 2H, OH, D2O exchangeable), 6.85- 6.97 (m, 6H, Ar-H), 7.88-7.92 (d, 2H, Ar-H)	281.6881

3e		3035 (Ar C-H), 1635, 1640 (C=O, α,β unsaturated), 1272 (C-N), 3490 (N-H), 1535 (C=C), 1289 (C-O), 3578 (O-H)	2.32 (s, 3H, CH3), 4.28 (s, 1H, NH, D2O exchangeable), 6.22-6.91 (m, 4H, Ar-H), 7.19-7.27 (d, 2H, Ar-H), 7.60-7.74 (dd, 2H, Ar-H), 7.96 (s, 1H, Ar-H)	263.9463
3f		3135 (Ar C-H), 16355, 1660 (C=O, α,β unsaturated), 1292 (C-N), 3570 (N-H), 1635 (C=C)	2.22 (s, 3H, CH3), 4.18 (s, 1H, NH, D2O exchangeable), 6.27-7.21 (m, 4H, Ar-H), 7.49-7.87 (d, 2H, Ar-H), 7.50-7.64 (dd, 2H, Ar-H), 7.76 (s, 1H, Ar-H)	277.1102
3g		3070 (Ar C-H), 1645, 1650 (C=O, α,β unsaturated), 1278 (C-N), 3495 (N-H), 1545 (C=C), 1299 (C-O), 3576 (O-H)	2.33 (s, 3H, CH_3), 4.29 (s, 1H, NH, D2O exchangeable), 6.23-6.92 (m, 4H, Ar-H), 7.29-7.37 (d, 2H, Ar-H), 7.65-7.79 (dd, 2H, Ar-H), 7.86 (s, 1H, Ar-H)	293.1051
3h		3028 (Ar C-H), 1632, 1637 (C=O, α,β unsaturated), 1253 (C-N), 3498 (N-H), 1525 (C=C), 3593 (O-H)	2.33 (s, 3H, CH3), 4.38 (s, 1H, NH, D2O exchangeable), 6.16-6.85 (m, 4H, Ar-H), 7.16-7.27 (d, 2H, Ar-H), 7.60-7.69 (dd, 2H, Ar-H), 7.94 (s, 1H, Ar-H)	265.7389

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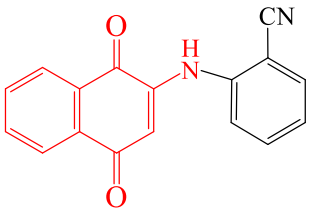
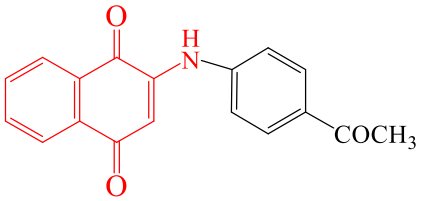
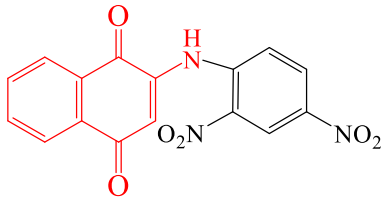
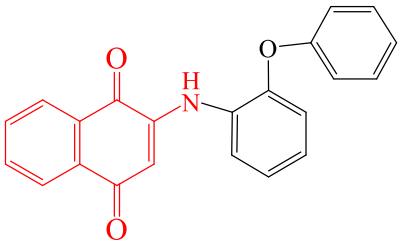
3i		3025 (Ar C-H), 1615, 1620 (C=O, α,β unsaturated), 1252 (C-N), 3490 (N-H), 1525 (C=C)	2.12 (s, 3H, CH3), 4.18 (s, 1H, NH, D2O exchangeable), 6.13-6.90 (m, 4H, Ar-H), 7.17-7.25 (d, 2H, Ar-H), 7.50-7.64 (dd, 2H, Ar-H), 7.94 (s, 1H, Ar-H)	
3j		3035 (Ar C-H), 1635, 1720 (C=O, α,β unsaturated), 1272 (C-N), 3590 (N-H), 1635 (C=C)	2.34 (s, 3H, CH3), 4.26 (s, 1H, NH, D2O exchangeable), 6.18-6.88 (m, 4H, Ar-H), 7.29-7.37 (d, 2H, Ar-H), 7.50-7.64 (dd, 2H, Ar-H), 7.66 (s, 1H, Ar-H)	294.6406
3k		3095 (Ar C-H), 1735, 1740 (C=O, α,β unsaturated), 1282 (C-N), 3520 (N-H), 1645 (C=C)	2.34 (s, 3H, CH3), 4.32 (s, 1H, NH, D2 O exchangeable), 6.13-6.83 (m, 4H, Ar-H), 7.15-7.23 (d, 2H, Ar-H), 7.58-7.72 (dd, 2H, Ar-H), 7.98 (s, 1H, Ar-H)	295.6670
3l		3095 (Ar C-H), 1735, 1740 (C=O, α,β unsaturated), 1282 (C-N), 3520 (N-H), 1645 (C=C)	2.36 (s, 3H, CH3), 4.31 (s, 1H, NH, D2O exchangeable), 6.25-6.94 (m, 4H, Ar-H), 7.21-7.29 (d, 2H, Ar-H), 7.62-7.76 (dd, 2H, Ar-H), 7.94 (s, 1H, Ar-H)	274.7423
			2.42 (s, 3H, CH3), 4.38 (s, 1H, NH, D2O exchangeable), 6.32-6.81 (m,	291.8954

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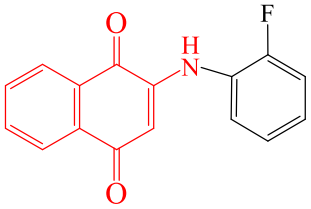
3m		3015 (Ar C-H), 1625, 1630 (C=O, α,β unsaturated), 1267 (C-N), 3477 (N-H), 1655 (C=C), 1589 (N-O)	4H, Ar-H), 7.14-7.32 (d, 2H, Ar-H), 7.64-7.78 (dd, 2H, Ar-H), 7.93 (s, 1H, Ar-H) 2.32 (s, 3H, CH_3), 4.29 (s, 1H, NH, D2O exchangeable), 6.23-6.93 (m, 4H, Ar-H), 7.21-7.29 (d, 2H, Ar-H), 7.58-7.71 (dd, 2H, Ar-H), 7.89 (s, 1H, Ar-H)	339.4914
3n		3085 (Ar C-H), 1715, 1720 (C=O, α,β unsaturated), 1252 (C-N), 3480 (N-H), 1654 (C=C), 1279 (C-O)	2.31 (s, 3H, CH3), 4.27 (s, 1H, NH, D2O exchangeable), 6.27-6.83 (m, 4H, Ar-H), 7.17-7.22 (d, 2H, Ar-H), 7.54-7.71 (dd, 2H, Ar-H), 7.96 (s, 1H, Ar-H)	341.1051
3o		3135 (Ar C-H), 1605, 1610 (C=O, α,β unsaturated), 1282 (C-N), 3530 (N-H), 1635 (C=C)		267.6956

Table 3
Analgesic activity of tested compounds (100 mg/kg b.w) and Nimesulide (50 mg/kg b.w).

Compound	Mean writhing (X±SE)	Protection (%)
Control	30.05±1.57	-
1	8.5±3.35	72.35
2	5.7±1.87	**81.35**
3	8.6±1.48	70.33
4	5.0±2.53	**83.38**
5	6.0±2.00	**80.00**
6	6.0±0.59	**80.22**
7	3.3±1.69	**89.00**
8	4.3±2.02	**85.77**
9	5.07±2.09	**83.34**
10	3.6±2.39	**88.46**
11	6.3±1.49	79.00
Nimesulide	-	100.00

Data represent mean values ± SE of six mice per group, shown at the final value for each group (saline, nimesulide, and tested compounds) after 3 h.

Data were analyzed using one-way ANOVA followed by Turkey–Krammer Multiple comparison test **p < 0.01.

Percentage change was calculated from basal (pre-drug) values.

And post-drug values. Protection was calculated as regards the percentage change of the Nimesulide. SE, standard error; Nim. Nimesulide.

The active compounds are marked in bold letters.

Table 4

Calculation of % Radical Scavenging and IC50 from DPPH assay				
Absorbance measurement data				
Concentration (µg/ml)	Control	Sample	%RSA	IC50
50	0.52	0.312	40	2.34
100	0.52	0.291	44.03846	7.73
150	0.52	0.228	56.15385	13.12
200	0.52	0.18	65.38462	18.51
250	0.52	0.14	73.07692	23.90
300	0.52	0.075	85.57692	29.29
350	0.52	0.035	93.26923	34.68

6.3.1. Animals used

For the purpose of researching acute toxicity, adult Swiss albino mice (20-25 g) and albino rats (150-200 g) of either sex were employed. In each group, six animals were housed individually in polypropylene cages with paddy husk beds. Animals were maintained at 25–27 C and 30–70% relative humidity. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC, Reg. No. 346/CPCSEA: Dated. 21-06-2022) before the experiment.

6.3.2. Analgesic activity screening

The morphine-induced writhing model was used to assess the palliative action of the synthesized compounds. Five groups of six Swiss albino mice, each 20–25 g b.w, were used. 0.6% morphine (dose ¼ 10 ml/Kg) was injected intra-peritoneally. The numbers of writhes were counted for 20 min, after 5 min of injection of morphine into each mouse. This reading was taken as a control. The next day, the same groups of mice were used for evaluating analgesic activity. Each group was administered orally with the synthesized compounds. The dose of 100 mg/kg of animal was given 1 hour before injection of morphine. After 5 min of morphine injection, mice were observed for the number of writhings for 20 min. The mean value for each group was calculated and compared with the control. Nimesulide was used as a standard drug for comparison of analgesic activity. Percent protection was calculated using the following formula:

(1-Vc/Vt)*100

Where

Vt = Mean number of writhing in test animals and Vc = Mean number of writhing in control. Statistical significance15 was analyzed using one-way ANOVA followed by Turkey–Krammer Multiple comparison tests and $p < 0.01$ was considered significant.

Antioxidant activity screening

DPPH-radical scavenging activity of synthesized compounds (3a-o) was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Solution of DPPH was prepared and was added to all the synthesized compounds (3a-o) at different concentrations (1-1000 mg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Among the tested compound (3d) 2-(2, 4-dihydoxyphenylamino) naphthalene-1, 4-dione showed pronounced antioxidant activity. All the analysis was made with the use of a UV-Visible Spectrophotometer (Shimadzu 1700). The absorbance of various concentrations was taken and percentage inhibition was calculated. Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity. Ascorbic acid was used as a standard antioxidant. IC50 (Inhibitory Concentration 50%) value denotes the concentration of sample required to scavenge 50% of the DPPH free radical. IC50 of all synthesized compounds (3a-o) was determined from the % Inhibition v/s concentration graph (Figure 1). The percentage discoloration was calculated as follows:

DPPH radical scavenging activity (%) = [AC517 – AE517 / AC517] x 100.

Where;

AC517 is the absorbance of a DPPH solution without fraction,

AE517 is the absorbance of the tested compounds with DPPH.

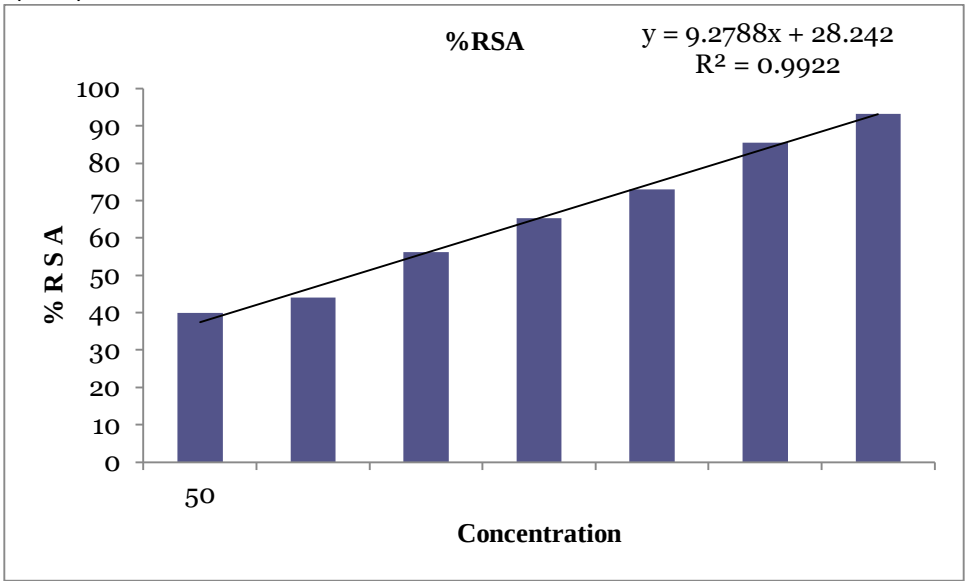


Figure 1 The graph shows that high activity in ascorbic acid (Std) then isolated compound

2.7. Statistical analysis

In the analgesic and antioxidant study, the results of the experiment were expressed as mean ± SEM. For group comparison, analysis of variance followed by Tukey's HSD multiple comparison test with SPSS version 10 was used. The difference among means was considered statistically significant when the p-value was less than 0.05.

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REFERENCES

1. O'Brien, P. J. Chem.-Biol. Interact. 1991, 80, 1-41.

2. Monks, T.J.; Hanzlik, R.P,: Cohen, G.M,: Ross, D,: Graham, D.G,; Toxicol. Appl. Pharmacol, 1992, 112, 2-16.

3. Babula, P,; Adam, V,; Havel, L,; Kizek, R. Ces. Slov. Farm., 2007, 56, 114-120.

4. Kim, B.H,; Yoo, J,; Park, S.H,; Jung, J.K,; Cho, H. Arch. Pharmacal Res., 2006, 29, 123-130.

5. Smith, M.T,; Evans, C.G,; Thor, H,; Sten, O. Ac. Pre.Inc., 1985 1–91.

6. Bialya, S.A.A,; Gouda, M.A.; J. Heterocycl. Chem, 2011, 48, 1280–1286.

7. Hamama, W.S,; Berghot, M.A,; Baz, E.A,; Gouda, M.A.; Arch. Pharm. , 2011, 344, 710-718.

8. Hamama, W.S,;, Zoorob, H.H,; Gouda, MA,; Afsah, E.M.;. Pharm. Chem. J., 2011, 45, 118-124.

9. Gouda, MA,; Abu-Hashem, A.A. Arch. Pharm, 2011, 344, 170-177.

10.Gouda, M.A,; Berghot, M.A,; Baz, E.A,; Hama ma, W.S. Med Chem, 2011, 21, 1062-1070.

11.Yogi, B,; Murti, Y,; Pathak, D. J. Pharm. Res, 2011, 4(10), 1-3.

12. Kokate, C. K. Val. Pra, 1994, 146–47.

13. Sieveking, I,; Thomas, P,; Estévez, J. C,; Quiñones, N,; Cuéllar, M.A,; Villena, J,; Espinosa-Bustos, C,; Fierro, A,; Tapia, R. A,; Maya, J.D,; López-Muñoz, R. Bioorg. Med. Chem. 2014 Sep 1;22(17):4609-20.

14. Koleva, II,; Van Beek, T. A,; Linssen, J.P,; Groot, A.D,; Evstatieva, L. N. Int. J. Chem. Technol.. 2002 Jan;13(1):8-17.

15. Uddin, S. N,; Ali, M. E,; Yesmin, M. N. Am. J. Plant Physiol.. 2010 (2):117-21.

16. Constantin, M,; Bromont, C,; Fickat, R,; Massingham, R. Biochem. Pharmacol. 1990 Oct 1;40(7):1615-22.

17. Matsubara, N,; Fuchimoto, S,; Iwagaki, H,; Nonaka, Y,; Kimura, T,; Kashino, H,; Edamatsu, R,; Hiramatsu, M,; Orita, K,; Pharmacol. Res. Commun.. 1991 Feb 1;71(2):239-42.

18. Yogi, B,; Mishra, A,; J. Pharm. Res. 2016,10(10),655-659.

19. Achar, K. C,; Hosamani, K. M,;. Eur. J. Med. Chem. 2010 May 1;45(5):2048-54.

20. Koster, R,; Anderson, M,; De Beer,; E.J. Fed. Proc. 18 (1952) 412–421.

21. Collier, H. D. J. Dinnin, L.C,; Johnson, C.A.,; Schneider, C. Br. J. Pharmacol. 32 (1968) 295–310.

22. Madkor, H.M.F,; Farag, A.A,; Sh Ramses, S,; Ibrahiem, N.A.A. J. Phosphorus Sulfur Silicon 181 (2006) 255–265

23. Roy, A,; Nishchaya, K,; Rai, V. K,;. Expert Opin Drug Deliv; .. 2022 Mar 4;19(3):303-19.

24. Kumar, N,; Kushwaha, K,; Swatantra, S,; Rai, K.;, Nanosci. Nanotechnol. - Asia 2021; 11(2) .