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Synthesis and biological evaluation of arylidene analogues of Meldrum's acid as a new class of antimalarial and antioxidant agents

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ABSTRACT

A series of arylidene analogues of Meldrum's acid were synthesized and evaluated for in vitro antimalarial and antioxidant activities for the first time. The influence of various physico-chemical parameters such as dielectric constant (ε), donor number (DN), acceptor number (AN), hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), and solubilizing power of the solvents on Meldrum's acid anion generation and thus on promoting the Knoevenagel condensation of Meldrum's acid with aryl aldehydes has been discussed. Five compounds **9l**, **9m**, **9n**, **9r**, and **9s** were found to be most active against *Plasmodium falciparum* with IC_{50} values in the range of 9.68–16.11 μM. Compound **9l** exhibited the most potent antimalarial activity (IC_{50} 9.68 μM). The compounds were also found to possess antioxidant activity when tested against DPPH and ABTS free radicals.

1. Introduction

Malaria is a tropical parasitic disease and one of the top three killers among communicable diseases.[1] It is a public health problem in more than 90 countries inhabited by about 40% of the world's population. According to recent WHO reports, it affects approximately 250 million people and kills about one million people every year.[2] Among four species of human malarial parasite, *Plasmodium falciparum* is considered most fatal due to high mortality rate particularly in children. Several drugs are being used in malaria-endemic regions of the world to control, treat, and prevent malaria; however, the emergence and spread of antimalarial drug resistance has made the malaria treatments ineffective and is therefore, a global problem.[3] Drug resistance can be defined as the ability of a parasite strain to survive and/or multiply in the presence of a drug administered in doses equal to or higher than those usually recommended.[4] Resistance of *P. falciparum* has emerged to all classes of antimalarial drugs (chloroquine (**1**), primaquine, sulfadoxine, pyrimethamine, etc.) except artemisinin (**2**)[5–8] (Fig. 1). The emergence of resistance of *P. falciparum* has provided a fresh impetus to the researchers for the development of a better and safe antimalarial drug. Recently, the use of antioxidants in the malaria as an adjunct treatment has generated a renewed interest. Several reports have evidenced that in patients with falciparum malaria infection oxidative stress is increased and relates to severity of disease and anemia.[9,10] Therefore, the antimalarial drugs with antioxidant action are of considerable interest due to their additional benefit to the treatment.

The various classes such as pyrimidines,[11] indole,[12] chalcone,[13] xanthone,[14] pyrazoline,[15] propenone,[16] etc., are reported to exhibit antimalarial activity. Licochalcone (**3**),[17] chalcones-like retinoid

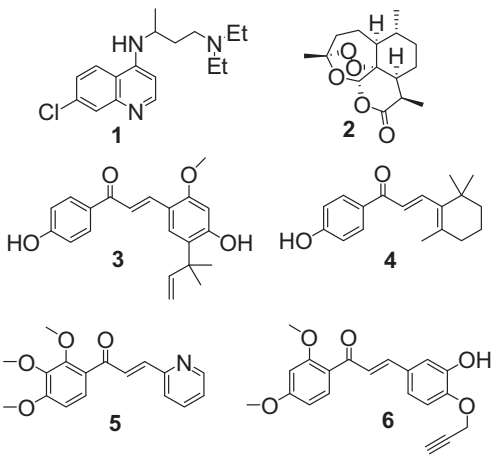

Figure 1. Chemical structures of antimalarial molecules.

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(**4**),[18] and various other chalcones (**5** and **6**)[16,19] (Fig. 1) are reported to possess promising antimalarial activity. It was noticed that chalcones with higher number of methoxyl groups exhibited potent antimalarial activity.

In view of the difficulty in making peroxides besides being more toxic it was thought worthwhile to design a compound which could react with Fe^{+2} of heme or free Fe^{+2} in a similar way, we conceptualized a molecule with structural features where important functionalities are equally placed as in artemisinin. The most suitable molecule we could design is in starting with an arylidene analogue of Meldrum's acid where two oxygen and one carbon atoms of the six membered ring of Meldrum's acid are exactly positioned with two oxygen and one carbon atoms of the six membered ring of artemisinin where one has to ignore the structural features while emphasizing on the shape of the molecule. We could visualize the ease of a ketal to bind with Fe^{+2} and proved to be correct (Fig. 2). The novelty of this molecule appears to be that site of the molecule where it binds with Fe^{+2} is symmetrical with the result that only one type radical is capable of interacting with Fe^{+2} while in artemisinin two types of radicals are possible.[7]

In the present communication the synthesis of various arylidene analogues of Meldrum's acid, the corresponding epoxides (Fig. 3) and their biological evaluation as antimalarial and antioxidant agents are reported for the first time.

2. Results and discussion

2.1. Optimization of reaction conditions for the synthesis of arylidene analogues of Meldrum's acid

Arylidene analogues (**9**) of Meldrum's acid are reported as the key intermediates for cycloaddition reactions and for the synthesis of heterocyclic compounds of biological importance such as cardiotonic[20a] and HIV integrase inhibitory activities.[20b] They are also reported to serve as the versatile substrates for various kinds of reactions.[21]

While planning for the synthesis of **9**, it was realized that a convenient synthetic protocol is lacking. The synthesis of **9** via Knoevenagel condensation of **7** with aldehydes has been documented either catalyzed by bases such as pyridine under reflux and water removal by Dean–Stark,[22a,b] piperidine/glacial acetic acid in benzene,[22c,d] piperidine[22e], pyrrolidine/acetic acid in dry benzene,[22f] NAP[22g] (3-aminopropylated silica gel), a potassium exchanged zirconium hydrogen phosphate $Zr(O_3POK)_2$ a heterogeneous basic catalyst[22h] or by Lewis acid such as anhydrous $ZnCl_2$,[23] or in ionic liquids,[24] or in solvents such as water[25] under heating for 2 h, DMF under heating at 80 °C[26,27] and DMSO,[27] or using surfactants[28] or under microwave irradiation,[29] or melt condition.[30] However, these methodologies have one or more disadvantages such as the use of high boiling solvent (e.g., DMF, DMSO, and benzene) that are difficult to recover, use of acid or base catalyst, special efforts required to prepare the catalysts (e.g., NAP and $Zr(O_3POK)_2$) or to prepare the polar medium (e.g., ionic liquids), need to use special apparatus (e.g., microwave irradiation) and heating conditions. Thus, there is necessity to develop a more effective and convenient synthetic procedure.

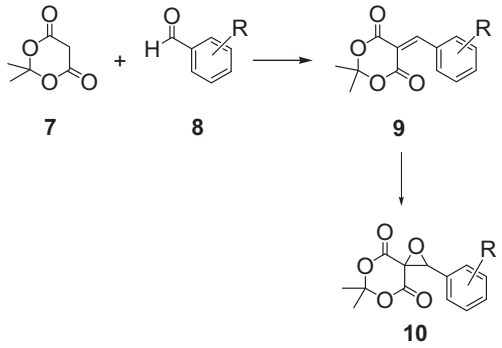


Figure 3. Proposed synthetic route of arylidene and epoxide derivatives of Meldrum's acid.

It is known that **7** exists in a boat form and its corresponding anion formed due to ease of deprotonation of one of the more acidic (flagpole) methylene hydrogens in a polar medium, is stabilized by extensive conjugation.[31] The reported condensation in polar medium[24–27,31] encouraged us to screen various solvents of different dielectric constants[32] (ε), (Scheme 1, Table 1).

In a model reaction, 3,4,5-trimethoxybenzaldehyde (**8i**, 1 mmol) was treated with **7** (1 mmol) under solvent-free conditions at rt (~30–35 °C) for 12 h that did not result in the significant formation (5%) of Knoevenagel product 2,2-dimethyl-5-(3,4,5-tri-

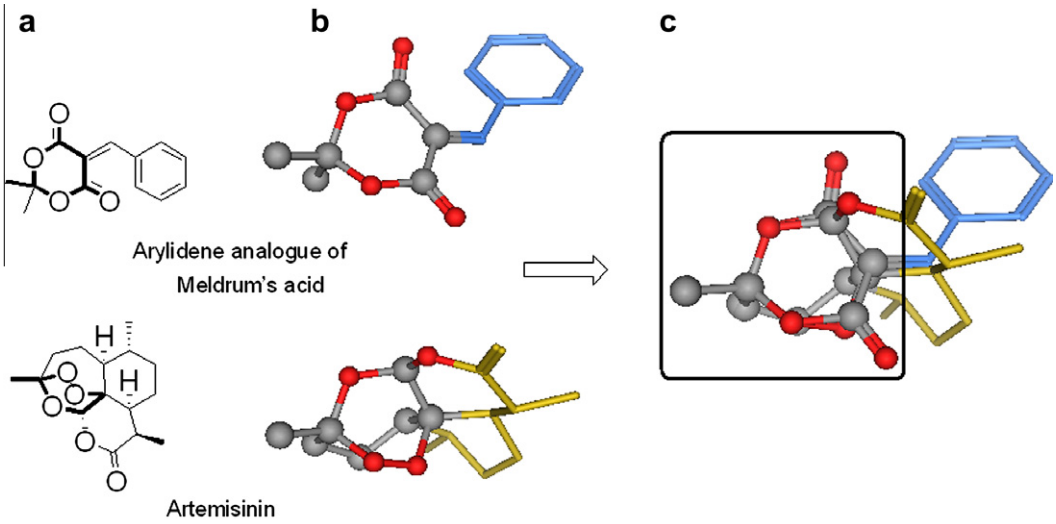
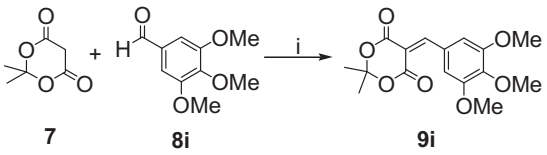


Figure 2. (a) Chemical structures of arylidene analogue of Meldrum's acid and artemisinin; (b) energy minimized structures of arylidene analogue of Meldrum's acid (blue) and artemisinin (yellow); and (c) overlay of arylidene analogue of Meldrum's acid and artemisinin (carbon: black, oxygen: red, overlay atoms are shown in ball). Hydrogens are removed for clarity.



Scheme 1. Reagents and conditions: (i) solvent, rt, 15 min.

Table 1
Knoevenagel condensation of **7** with **8i**[a]

Entry	Solvent	ε[b]	DN[c]	AN[c]	HBD (α)[d]	HBA (β)[e]	Yield[f] (/ %)
1	Neat						5 (15)[g]
2	PhMe	2.4	—	—	—	—	10
3	Hexane	1.9	—	0.0	—	—	13
4	$CHCl_3$	4.8	—	23.1	Nil	—	17
5	DCM	8.9	—	20.4	—	—	19
6	DCE	10.3	0.0	16.7	—	—	23
7	EtOAc	6.0	17.1	9.3	—	0.481	21
8	MeCN	36.6	14.1	18.9	0.29	—	32
9	1,4-Dioxane	2.2	14.8	10.8	—	0.386	18
10	Et_2O	4.3	19.2	3.9	—	0.488	24
11	THF	7.6	20.0	8.0	—	0.523	37
12	DMF	38.3	26.6	16.0	—	0.710	48 (65)[g]
13	DMSO	47.2	29.8	19.3	—	0.752	51 (71)[g]
14	MeOH	32.7	20.0[b]	41.5	0.990	0.70	96
15	Dry MeOH					15	93
16	EtOH	24.6	19.0[b]	37.1	0.850	0.73	88
17	^{i}PrOH	9.9	—	33.5	0.687	0.80	82
18	H_2O	80	—	54.8	1.017	0.47	35 (66)[g]

[a] **8i** (1 mmol) was treated with **7** (1 mmol) in the solvent (1 mL) at room temperature (30–35 °C).
[b] Values were taken from Ref. 32a.
[c] Values were taken from Ref. 33.
[d] Values were taken from Ref. 34.
[e] Values were taken from Ref. 35.
[f] Determined by IR and NMR.
[g] The figure in parenthesis is the yield obtained after heating the reaction mixture for 2 h at 80 °C.

methoxybenzylidene)-[1,3]-dioxane-4,6-dione (**9i**) (Scheme 1), indicating the requirement of a solvent for the reaction.

The reactions were performed in solvents of different polarity. The importance of solvent polarity was demonstrated by the observations that excellent results were obtained in MeOH having the highest value of ε (except water, MeCN, DMF and DMSO) of all the solvents used at rt after 15 min (96%) (Table 1). The product yield decreased with the decrease of ε of the alcohols and a similar trend was also observed for the ethereal solvents. The Knoevenagel adduct **9i** was formed in 18%, 24%, and 37% yields after 15 min in 1,4-dioxane, Et_2O, and THF, respectively. The poor yields were obtained using the hydrocarbon and halogenated solvents due to their low ε values and low solubility of starting materials in them. The use of MeOH under non-anhydrous and anhydrous conditions resulted in comparable yields and provided an added advantage to this new methodology in eliminating additional efforts required in drying the solvent.

Lesser yields obtained with the use of MeCN, DMF, DMSO, and water signified the role of various factors other than ε in promoting the condensation (Table 1). The involvement of various factors such as donor number[33] (DN), acceptor number[33] (AN), hydrogen bond donor[34] (HBD, α), and hydrogen bond acceptor[35] (HBA, β) of the solvents and their influence in Meldrum's acid anion generation and hence on Knoevenagel condensation was also considered. Solvent MeCN, in spite of having higher ε (36.6) than MeOH (32.7), afforded only 32% product which could be probably due to its low DN, poor AN, poor HBD, and nil HBA. Similarly, DMF and DMSO have higher ε, higher DN but lesser AN, comparable HBA, and nil HBD than MeOH; whereas the water has highest ε, higher AN, comparable HBD but lesser DN (data not reported), lesser HBA and poor solubility of starting materials at rt and hence retarded the rate of reaction. This was further supported by the fact that the formation of the condensed product was enhanced from 35% to 66% and 68% while carrying out the reaction under reflux for 2 h or in surfactant (sodium dodecyl sulfate; 100 mol %) at rt for 2 h, respectively, indicating the influence of solubility. The results were found in good agreement with the previously reported studies.[25,28]

Thus we can anticipate that the high ε of MeOH makes it a polar medium with the ability to donate hydrogen bond (HBD), accept hydrogen bond (HBA), optimum Lewis acidity (AN 41.5), Lewis basicity (DN 20.0) and solubility of the starting materials at rt during Knoevenagel condensation make it an ideal coordinating solvent with the **7** and that results into Meldrum's acid anion generation **11** via a polar six membered transitions state **TS** (Scheme 2) followed by condensation with aldehyde **8** to yield the dehydrated product **9** as the target compound.

2.2. Synthesis of arylidenes and the corresponding epoxides

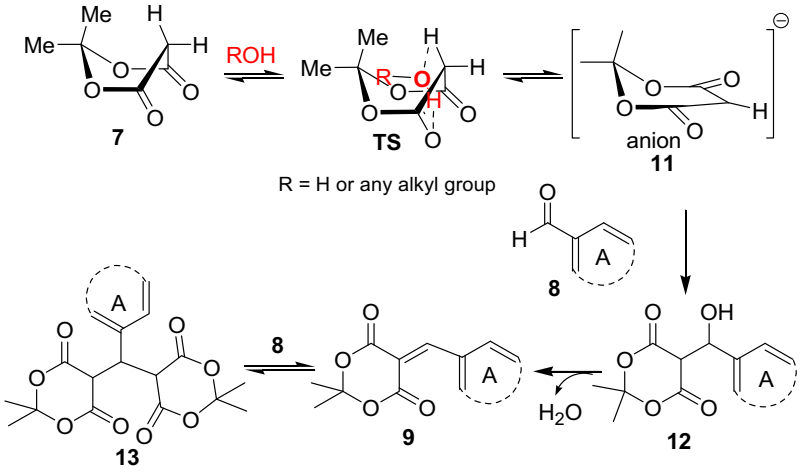
By using optimized procedure, we synthesised compounds **9a** and **9d–w** (85–96%) via Knoevenagel condensation of **7** (1 equiv) with aldehydes (**8a** and **8d–w**; 1 equiv) in MeOH at rt (Scheme 3, Fig. 4) in 15–45 min. Various aromatic and heteroaromatic aldehydes condensed smoothly. The crude products were purified by column chromatography and were characterized by using spectroscopic techniques such as IR, NMR, and mass spectrometry. The reaction was found to be compatible with diverse functional groups such as ether, nitro, hydroxyl and halogens.

It was noticed that the reaction of **7** with aldehydes bearing electron withdrawing group(s) irrespective of their position on aromatic ring (**8b** and **8c**) in MeOH yielded a mixture of Knoevenagel product (**9b** and **9c**), aldol product (**12b** and **12c**) and Michael adduct (**13b** and **13c**) (Scheme 2). Similar results were obtained in carrying out the reaction with water under heating for 2 h which were in accordance with earlier observations reported by Bigi et al.[25] The reason why electron donating substituents on aromatic ring accelerate the last step of Knoevenagel condensation and electron withdrawing groups accelerate the first step of Knoevenagel condensation is still unclear and is a question of study.[22e,25] Though the various approaches such as methoxide,[36] amines,[37] and thiols[38] are utilized to minimize the formation of Michael adduct **13** but this is an unsatisfactory solution in terms of atom and step economy. However we succeeded to synthesize the desired compounds **9b** and **9c** with excellent selectivity (>96) when the reaction of **7** (1 equiv) with **8b** and **8c** (1 equiv each) was carried out separately under the catalytic influence of type 3 Å MS (50 mol %) in MeOH under reflux for 3 h. Two epoxide derivatives **10a** and **10b** (Scheme 4, Fig. 4) and one 2-benzylidenecyclohexane-1,3-dione (**14**), respectively, were synthesized as per the reported procedures.[39,40]

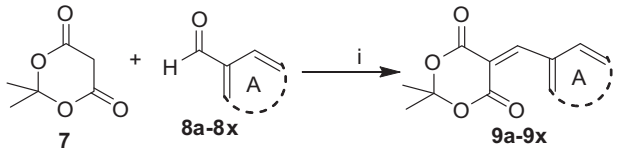
2.3. Biological evaluation of synthesized compounds for antimalarial activity

In vitro screening of all the compounds (**9a–x**, **10a**, **10b**, and **14**) was carried out for antimalarial activity using histidine-rich protein 2 (HRP-2) double site sandwich enzyme-linked immunosorbent assay (ELISA) with the previously reported method.[41] Each compound was tested in triplicate. None of the compounds showed any cytotoxic activity (IC_{50} value higher than 100 μM after 48 h).

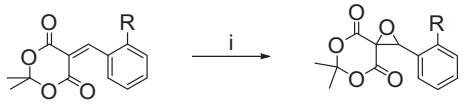
Among the series of 27 compounds, five compounds **9l**, **9m**, **9n**, **9r**, and **9s** were found to be most active against the resistant strain of *P. falciparum* with IC_{50} ranging from 9.68 μM to 16.11 μM



Scheme 2. Knoevenagel condensation of **7** with **8**.



Scheme 3. Reagents and conditions: (i) For **9a** and **9d**–**9w**: MeOH, rt, 15–45 min, 85–96% and for **9b** and **9c**: MeOH, 3 Å MS, reflux, 3 h, 77–81%.



Scheme 4. Reagents and conditions: (i) H_2O_2, MeCN, rt, 10 min to 1 h, 58–62%.

(Table 2). Compound **9l** exhibited the most potent antimalarial activity (IC_{50} 9.68 μM).

2.4. Structure–activity relationship (SAR)

The compounds bearing electron withdrawing substituents on phenyl moiety of ring A irrespective of their position, were found to be inactive or less active than electron donating substituents (**9b**, **9c**, and **9d**–**p**). Replacement of phenyl group with more bulky groups such as 1-naphthyl (**9s**) and 2-naphthyl (**9r**), resulted in enhancement of antimalarial activity (except 2-methoxy-1-naphthyl; **9u** and anthranyl; **9t**). The compounds with hydroxyl group on phenyl ring at *p*-position (**9l**) showed better activity than those with *m*-position (**9n**). The replacement of the phenyl ring with 2-theinyl (**9v**) or 3-indolyl (**9w**) did not result in increase of the activity. The conversion of the arylidene analogues into their

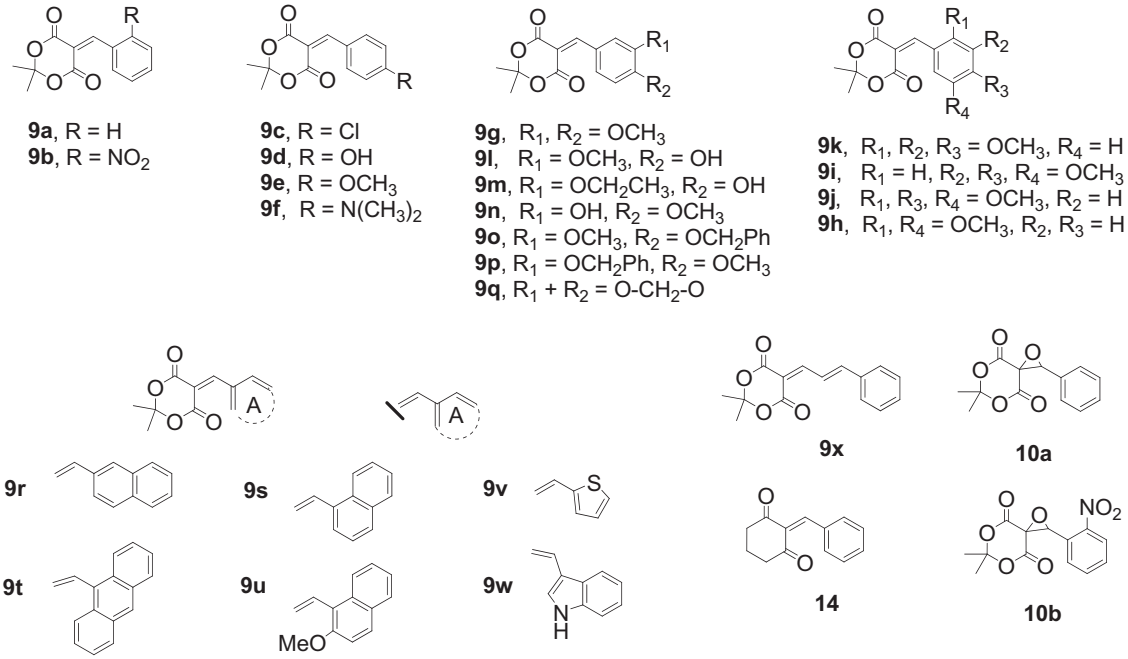


Figure 4. Chemical structures of the synthesized compounds.

Table 2
In vitro screening of the synthesized compounds (**9a–9x**, **10a–10b** and **14**) for antimalarial activity

Compound	Antimalarial activity	*P. falciparum*[a] (IC_{50}, μM)
9a		37.82
9b		39.63
9c		>100
9d		34.64
9e		36.37
9f		35.21
9g		26.05
9h		33.20
9i		20.21
9j		33.94
9k		34.68
9l		9.68
9m		15.54
9n		12.44
9o		37.21
9p		36.06
9q		28.09
9r		14.34
9s		16.11
9t		>100
9u		31.84
9v		>100
9w		41.72
9x		24.31
10a		>100
10b		>100
14		>100
1	Chloroquine	1.03
2	Artemisinin	2.8 nM

[a] Values are means of three experiments.

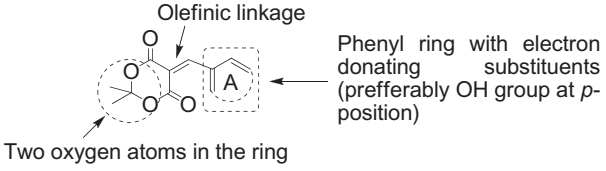


Figure 5. Essential structural requirements for antimalarial activity.

corresponding epoxides (**9a** and **9b)** resulted in loss of the antimalarial activity indicating the importance of olefinic linkage. Further no activity was observed with **14** highlighting the significance of two oxygen atoms. These observations helped us to formulate a basic pharmacophore with the structural features required for antimalarial activity (Fig. 5).

2.5. Biological evaluation for antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals[42,43] are commonly used for assessing the antioxidant property of substances of natural or synthetic origin because of their good reproducibility. Both assays measure the decrease in absorbance of radicals at a characteristic wavelength during the reaction with antioxidant; which reverses the formation of ABTS radical cation and DPPH radical:

$$DPPH^{\cdot} + AH \rightarrow DPPH + A^{\cdot}$$

$$ABTS^{\cdot+} + AH \rightarrow ABTS^{+} + A^{\cdot}$$

It is well known that ABTS activity is closely related with DPPH[44] because both are responsible for the same chemical property of hydrogen or electron donation.

Table 3
Antioxidant activity of synthesized compounds[a]

Compound	Antioxidant activity			
	(% Inhibition at 100 μM)[a]		(IC_{50}, μM)	
	DPPH	ABTS	DPPH	ABTS
9b	6.41 ± 1.11	1.30 ± 0.26	—	—
9d	5.12 ± 0.76	14.40 ± 1.46	—	—
9e	5.81 ± 0.37	9.26 ± 1.64	—	—
9f	10.32 ± 1.45	13.89 ± 0.62	—	—
9g	4.74 ± 0.38	6.84 ± 0.61	—	—
9h	12.17 ± 0.12	13.79 ± 1.30	—	—
9i	3.24 ± 0.89	5.33 ± 0.82	—	—
9j	6.23 ± 0.47	9.86 ± 0.53	—	—
9k	5.72 ± 1.79	12.99 ± 1.66	—	—
9l	76.02 ± 0.12	92.59 ± 1.60	23.99	30.48
9m	66.15 ± 1.11	95.56 ± 0.78	39.10	30.65
9n	40.25 ± 1.03	52.76 ± 2.54	80.03	72.16
9o	10.68 ± 1.58	3.62 ± 0.90	—	—
9q	8.20 ± 0.51	8.25 ± 0.70	—	—
9r	20.62 ± 1.41	17.01 ± 1.02	—	—
9s	8.88 ± 0.37	7.55 ± 0.52	—	—
9t	8.76 ± 0.45	10.67 ± 0.56	—	—
9u	9.48 ± 2.82	4.73 ± 0.66	—	—
9v	6.32 ± 0.42	3.77 ± 1.60	—	—
9w	1.79 ± 0.77	3.12 ± 0.78	—	—
9x	53.07 ± 0.97	20.24 ± 1.84	—	—
Trolox	99.48 ± 1.05	99.12 ± 0.09	15.72	16.23
AA	99.43 ± 0.12	98.03 ± 1.17	22.44	25.52

[a] Values are expressed as means ± SEM of three independent experiments.

Table 4
Antiradical power and stoichiometry from the DPPH assay

Compound	Parameters of DPPH assay			
	ED_{50}	ARP	Stoichiometric value	H atoms per molecule
9l	0.39	2.56	0.78	1.23
9m	0.65	1.53	1.30	0.76
9n	1.33	0.75	2.66	0.37
Trolox	0.26	3.84	0.52	1.92
Ascorbic acid	0.37	2.70	0.74	1.35

2.5.1. Free radical scavenging effects in DPPH assay

The free radical scavenging effect of the synthesized compounds were tested in DPPH assay.[45,46] Initially, all the compounds were tested at 100 μM concentration and their results are shown in Table 3. Three compounds **9l**, **9m**, and **9n** were found to be strong free radical scavengers with 76.0%, 66.1%, and 40.2% scavenging of the DPPH activity. All other compounds were found to have very weak radical scavenging activity. The study was further extended to determine the scavenging effects of **9l**, **9m**, and **9n** at different concentrations and to calculate the IC_{50} values and various other parameters (Table 3 and Table 4).

Compound **9l** was found to exhibit comparable IC_{50} and ED_{50} values (Table 4) with that of ascorbic acid while it was less active than that of trolox. Compounds **9m** and **9n** were less active than **9l**. Compound **9l** was found to exhibit the reduction of more than one molecule of DPPH per molecule of **9l**; whereas **9m** and **9n** demonstrated the reduction of less than one molecule of DPPH/molecule. The standard trolox demonstrated the reduction of about two molecules of DPPH/molecule. Thus, it can be concluded that compounds **9l** showed the comparable activity as that of ascorbic acid.

2.5.2. Free radical scavenging effects in ABTS assay

$ABTS^{\cdot+}$ cation radical is commonly used to determine the hydrogen-donating ability of antioxidants.[47,48] Initially, all the compounds were tested at 100 μM concentration and their results are shown in Table 3. The similar results were also obtained in this assay. Compound **9l**, **9m**, and **9n** showed the 92.5%, 95.5%, and 52.7%

scavenging of the ABTS free radical, respectively; while the other compounds were weak radical scavengers. The IC_{50} values of **9l**, **9m**, and **9n** were found as 30.4, 30.6, and 72.1 μM, respectively (Table 4).

3. Conclusions

The present study was initiated with the aim of providing an efficient and convenient method for the synthesis of arylidene analogues of Meldrum's acid. The optimization of the synthetic procedure was carried out and the effect of the various factors such as donor number (DN), acceptor number (AN), hydrogen bond donor (HBD, α), and hydrogen bond acceptor (HBA, β) and the solubility on the reaction conditions was also studied. The MeOH emerged as the best suitable solvent for carrying out the Knoevenagel condensation of Meldrum's acid with aldehydes. Following the optimized procedure twenty four analogues of Meldrum's acid were synthesized out of which six were found as new. The synthesized compounds were evaluated for antimalarial (against *P. falciparum*) and free radical (DPPH and ABTS) scavenging activities. SAR study revealed that: (i) nature of substituents present on phenyl ring, (ii) an olefinic double bond, and (iii) two oxygen atoms are critical requirements for high antimalarial activity. Further the determination of mode of action of arylidene analogues of Meldrum's acid and a detailed target analysis is currently in progress.

4. Experimental

The reagents were purchased from Sigma–Aldrich, Loba and CDH, India and used without further purification. All yields refer to isolated products after purification. Products were characterized by comparison with authentic samples and by spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR spectra). The NMR spectra were recorded on a Bruker Avance DEX 400 MHz instrument. The spectra were measured in $CDCl_3$ relative to TMS (0.00 ppm). IR (KBr pellets) spectra were recorded on a Fourier transform infrared (FT-IR) Thermo spectrophotometer. Melting points were determined in open capillaries and were uncorrected.

4.1. Typical experimental procedure for the synthesis of 9a and 9d–w: 2,2-Dimethyl-5-(3,4,5-trimethoxybenzylidene)-[1,3]dioxane-4,6-dione (9i)[21d]

A mixture of **7** (1 mmol, 1 equiv, 0.14 g) and **8i** (1 equiv, 0.19 g) in methanol (1 mL) was stirred at room temperature. The progress of the reaction was monitored by TLC (15 min). After the completion of reaction, the precipitate was filtered-off and dried to afford **9i** (0.30 g, 96%). The remaining reactions were carried out following these general procedures. In each occasion, the spectral data (IR, ^{1}H NMR and ^{13}C NMR) of known compounds such as 5-benzylidene-2,2-dimethyl-[1,3]dioxane-4,6-dione (**9a**),[28] 5-benzylidene-2,2-dimethyl-[1,3]dioxane-4,6-dione (**9a**),[28] 5-(4-hydroxybenzylidene)-2,2-dimethyl-[1,3]dioxane-4,6-dione (**9d**),[25] 5-(4-methoxybenzylidene)-2,2-dimethyl-[1,3]dioxane-4,6-dione(**9e**),[25] 5-(4-dimethylaminobenzylidene)-2,2-dimethyl-[1,3]dioxane-4,6-dione (**9f**),[24a] 5-(3,4-dimethoxybenzylidene)-2,2-dimethyl-[1,3]dioxane-4,6-dione (**9g**),[24a] 5-(4-hydroxy-3-methoxybenzylidene)-2,2-dimethyl-[1,3]dioxane-4,6-dione (**9l**),[22f] 5-(3-hydroxy-4-methoxybenzylidene)-2,2-dimethyl-[1,3]dioxane-4,6-dione (**9n**),[20b] 5-benzo[1,3]dioxol-5-ylmethylene-2,2-dimethyl-[1,3]dioxane-4,6-dione (**9q**),[22c] 5-(2,3,4-trimethoxybenzylidene)-2,2-dimethyl-[1,3]dioxane-4,6-dione (**9k**),[21e] 5-(2,4,5-trimethoxybenzylidene)-2,2-dimethyl-[1,3]dioxane-4,6-dione (**9j**),[21e] 2,2-dimethyl-5-naphthalen-2-ylmethylene-[1,3]dioxane-4,6-dione (**9r**),[22f] 2,2-dimethyl-5-naphthalen-1-ylmethylene-[1,3]dioxane-4,6-dione (**9s**),[22f] 2,2-dimethyl-5-thiophen-2-ylmethylene-[1,3]dioxane-4,6-dione (**9v**),[21d] 5-(1*H*-Indol-3-ylmethylene)-2,2-dimethyl-[1,3]dioxane-4,6-dione (**9w**),[20b] 2,2-dimethyl-5-(3-phenyl-allylidene)-[1,3]dioxane-4,6-dione (**9x**),[25] 6,6-dimethyl-2-phenyl-1,5,7-trioxaspiro[2.5]octane-4,8-dione (**10a**),[39] 6,6-dimethyl-2-(2-nitro-phenyl)-1,5,7-trioxaspiro[2.5]octane-4,8-dione (**10b**),[39] 2-benzylidenecyclohexane-1,3-dione (**14**),[40] were found to be identical with those reported in the literature. The physical data of new compounds are provided below.

4.1.1. 5-Anthracen-9-ylmethylene-2,2-dimethyl-[1,3]dioxane-4,6-dione (9t)

Yellow solid; mp 182–184 °C; IR (KBr): ν_{max} 1732, 1596, 1363 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.91 (s, 6H), 7.52 (m, 4H), 7.82 (m, 2H), 8.06 (m, 2H), 8.55 (s, 1H), 9.47 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.15, 104.94, 121.03, 124.50, 125.56, 125.71, 126.80, 127.06, 128.63, 129.32, 130.25, 130.94, 157.72; MS-ESI: m/z 355.07 [M+Na]$^+$. Anal. Calcd for $C_{21}H_{16}O_4$: C, 75.89; H, 4.85. Found: C, 76.12; H, 5.01.

4.1.2. 5-(2,5-Dimethoxybenzylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (9h)

Yellow solid; mp 104–106 °C; IR (KBr): ν_{max} 1726, 1585, 1377 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.81 (s, 6H), 3.80 (s, 3H), 3.85 (s, 3H), 6.87 (d, 1H, J = 9.12 Hz), 7.08 (dd, 1H, J_{12} = 3.12 Hz, J_{13} = 9.12 Hz), 7.68 (d, 1H, J = 3.12 Hz), 8.73 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.51, 55.89, 56.08, 104.43, 112.03, 115.04, 116.14, 121.49, 122.12, 152.38, 152.87, 154.41, 160.10, 163.32; MS-ESI: m/z 314.93 [M+Na]$^+$. Anal. Calcd for $C_{15}H_{16}O_6$: C, 61.64; H, 5.52. Found: C, 61.39; H, 5.87.

4.1.3. 5-(3-Ethoxy-4-hydroxybenzylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (9m)

Yellow solid; mp 84–87 °C; IR (KBr): ν_{max} 3366, 1748, 1698, 1546 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.5 (t, 3H, J = 6.92 Hz), 1.79 (s, 6H), 4.23 (q, 2H, J = 6.92 Hz), 6.45 (OH, D_2O exchangeable, 1H), 7.01 (d, 1H, J = 8.24 Hz), 7.54 (d, 1H, J = 8.24 Hz), 8.35 (s, 1H), 8.39 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.63, 27.47, 64.88, 104.16, 110.14, 114.52, 116.05, 124.73, 133.41, 145.59, 152.22, 158.66, 160.80, 164.23; MS-ESI: m/z 315.20 [M+Na]$^+$. Anal. Calcd for $C_{15}H_{16}O_6$: C, 61.64; H, 5.52. Found: C, 61.33; H, 5.76.

4.1.4. 5-(2-Methoxy-naphthalen-1-ylmethylene)-2,2-dimethyl-[1,3]dioxane-4,6-dione (9u)

Yellow solid; mp 177–179 °C; IR (KBr): ν_{max} 1732, 1597, 1364 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.91 (s, 6H), 3.97 (s, 3H), 7.29 (d, 1H, J = 9.16 Hz), 7.44 (m, 1H), 7.57 (m, 1H), 7.83 (m, 2H), 7.98 (d, 1H, J = 9.16 Hz), 8.84 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.48, 56.04, 104.67, 112.66, 115.61, 119.56, 123.39, 124.60, 128.22, 128.52, 128.74, 132.36, 134.06, 149.64, 155.72, 159.99, 162.91; MS-ESI: m/z 335.00 [M+Na]$^+$. Anal. Calcd for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.41; H, 5.51.

4.1.5. 5-(3-Methoxy-4-benzyloxybenzylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (9o)

Yellow solid; mp 186–188 °C; IR (KBr): ν_{max} 1701, 1596, 1364 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.79 (s, 1H), 3.99 (s, 3H), 5.23 (s, 2H), 6.97 (d, 1H, J = 8.60 Hz), 7.32–7.51 (m, 5H), 7.65 (dd, 1H, J_{12} = 2.12 Hz, J_{13} = 8.60 Hz), 8.30 (d, 1H, J = 2.12 Hz), 8.32 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.48, 56.23, 70.81, 104.18, 110.61, 110.87, 117.68, 124.52, 127.66, 128.08, 128.57, 132.77, 136.33, 147.79, 155.32, 158.23, 160.64, 164.15; MS-ESI: m/z 391.00 [M+Na]$^+$. Anal. Calcd for $C_{21}H_{20}O_6$: C, 68.47; H, 5.47. Found: C, 68.24; H, 5.74.

4.1.6. 5-(3-Benzyloxy-4-methoxybenzylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (9p)

Yellow solid; mp 174–176 °C; IR (KBr): ν_{max} 1703, 1594, 1363 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.79 (s, 6H), 3.97

(s, 3H), 5.29 (s, 2H), 6.95 (d, 1H, J = 8.52 Hz), 7.34–7.45 (m, 5H), 7.59 (dd, 1H, J_{12} = 2.12 Hz, J_{13} = 8.52 Hz), 8.30 (d, 1H, J = 2.12 Hz), 8.35 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.49, 56.07, 70.85, 104.19, 110.70, 112.38, 116.07, 125.23, 127.19, 128.30, 128.78, 132.33, 135.76, 149.14, 153.87, 158.22, 160.64, 164.17; MS-ESI: m/z 391.10 $[M+Na]^+$. Anal.Calcd for $C_{21}H_{20}O_6$: C, 68.47; H, 5.47. Found: C, 68.31; H, 5.82.

4.2. Typical experimental procedure for the synthesis of 9b and 9c

To a mixture of **7** (1 mmol, 1 equiv, 0.14 g) and **8b** (1 equiv, 0.15 g) in methanol (1 mL) was added MS 3 Å (50 mol %). The reaction mixture was refluxed for 3 h (TLC) The reaction mixture was filtered, dried under reduced pressure and washed with ether and hexane mixture to afford pure **9b** (0.22 g, 81%). Mp 116–118 °C (Lit.[24a] 117–118 °C).

4.3. Biology

4.3.1. Antimalarial assay

4.3.1.1. *P. falciparum* in vitro culture. Parasites were maintained in in vitro culture using the method of Trager and Jensen.[41a] Briefly, an equal volume of sterile 3.5% sodium chloride was added to the infected blood samples, and the mixture was centrifuged at 1000*g* for 5 min. The pellet was washed three times and re-suspended with RPMI-1640 medium supplemented with 10% human serum and placed in a 25 mL tissue culture flask in a total volume of 10 mL containing a 5% RBC suspension. The flask was flushed with a gas mixture of 5% CO_2, 5% O_2, and 90% N_2 for 30 s and incubated at 37 °C. The culture medium was changed once a day; group O red blood cells were added to maintain the 5% cell suspension. Parasite growth was monitored by thin smear examination with Field stain (A and B).

4.3.1.2. Hrp2 ELISA. A commercial HRP2 ELISA kit[41b] was used for the quantification of HRP2 in the culture samples. One hundred microliters of each of the hemolyzed culture samples was transferred to the ELISA plates, which are precoated with monoclonal antibodies against *P. falciparum* HRP2 (capture antibody of the immunoglobulin M class; code CPF4), and the plates were incubated at room temperature for 1 h. Subsequently, the plates were washed four times with the washing solution provided with the test kit, and 100 μL of the diluted antibody conjugate (an indicator antibody of the immunoglobulin G1 isotype; code CPF6) was added to each well. After incubation for an additional 1 h, the plates were washed four times and 100 μL of diluted (1:20) chromogen (tetramethylbenzidine) was added to each well. The plates were then incubated for another 15 min in the dark, and 50 μL of the stock solution was added. Spectrophotometric analysis was performed with a Multiscan Ex ELISA reader (Thermo Scientific) at an absorbance maximum of 450 nm. The optical density values correspond to the amount of HRP2 found in the culture samples and provide consistent indicators of parasite growth.[41c]

4.3.2. Antioxidant assay

4.3.2.1. DPPH radical scavenging assay. The free radical scavenging activities of compounds were determined by the widely used 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay employing trolox as reference standard.[45,46] Briefly, to a methanolic solution of DPPH (60 μM, 3.9 mL) was added the different concentrations of test compound (0.1 mL), the resulting mixture was incubated at room temperature for 1 h and then the absorbance was read at 517 nm. The experiment was done in triplicate for each concentration and the results are expressed as the% of the DPPH radical scavenged by the test compounds. The IC_{50} is defined as the concentration of the test compound that causes 50% scavenging of DPPH radical and was calculated from the regression analysis obtained by plotting the scavenging effect of compounds at five different concentrations. ED_{50}, efficient dose is defined as micromoles of compound able to consume half the amount of free radical divided by micromoles of initial DPPH and the value is obtained by dividing IC_{50} value by 60. The inverse of ED_{50} is the measure of the antiradical power (ARP). By multiplying the ED_{50} by two, the stoichiometric value (theoretical concentration of antioxidant to reduce 100% of the DPPH) is obtained. The inverse of this value represents the moles of DPPH reduced by one mole of antioxidant and gives an estimate of the number of hydrogen atoms involved in the process.

4.3.2.2. ABTS radical scavenging assay. 2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging activity of the compounds were determined using an ABTS radical cation decolorization assay.[47,48] ABTS was dissolved in water to a concentration of 7 mM. The $ABTS^{\cdot+}$ cation radical was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and by allowing the mixture to stand in the dark for 12 h. To an aliquot (5 μL) of ethanol solution of test compounds was added ABTS solution (0.1 mL) and the absorbance was read at 734 nm after mixing up to 6 min. All the determinations were carried out in triplicate and the results are expressed as the% of the ABTS radical scavenged by the test compounds. The IC_{50} values were calculated by regression analysis in the similar manner as described above.

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