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Synthesis and characterization of some novel sulfonate and carbonate prodrugs of Atovaquone, accomplished with better solubility profile

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Abstract

Atovaquone is an acclaimed anti-malarial drug with considerably poor water solubility and hence has low bioavailability. To overcome this issue, a few novel prodrugs of Atovaquone were introduced in this work. In line with this, two Atovaquone prodrugs with sulfonate and carbonate molecular framework extensions were disclosed. This work encapsulates the synthesis, characterization, solubility profile, and the feasibility of hydrolysis of disclosed prodrugs. These prodrugs have shown better water solubility than the parent drug, Atovaquone. Hence, prodrugs of the present invention can contribute to increase the bioavailability and clinical efficacy of Atovaquone. It is worth perusing further *in-vivo* studies with these prodrugs to solidize them for routine clinical practices with safe and efficacious drug delivery.

Keywords: Atovaquone; sulfonate prodrugs; carbonate prodrugs; solubility; hydrolysis; metabolites.

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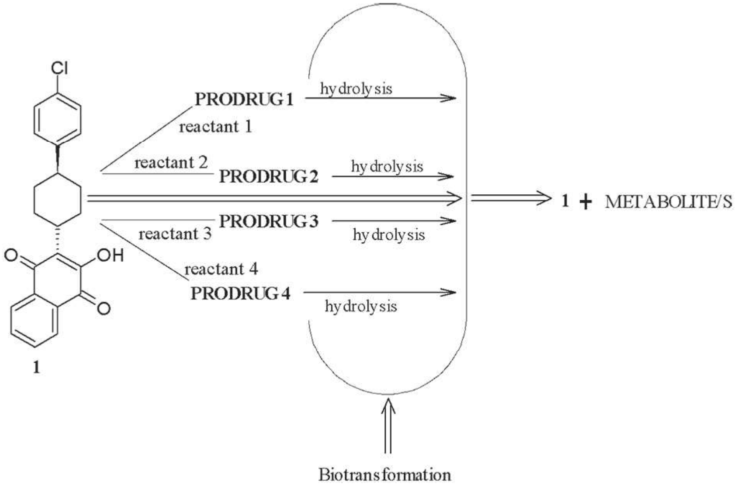
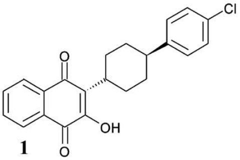
Graphical Abstract



1. Introduction

Atovaquone **1** has the IUPAC name 2-[*trans*-4-(4-chlorophenyl) cyclohexyl]-3-hydroxynaphthalene-1,4-dione ($C_{22}H_{19}ClO_3$) with the trade name Mepron or Wellvone (CAS Register No:- 95233-18-4). It is an analogue of Lawsone, predominantly used as a medication against *Pneumocystis pneumonia* (PCP) caused by the fungus *Pneumocystis jirovecii* [1, 2]. It is used as a medication to treat for the *Toxoplasmosis* [3]. To treat *Malaria*, it is combined with Proguanil to form a combinational drug (Malarone) [4]. Furthermore, it is often used for the treatment of *Babesia* along with oral Azithromycin [5-7]. A certain therapeutic bottleneck of **1** is its poor bioavailability due to solubility issues. Low water solubility of **1** can be attributed to its intense lipophilicity [8, 9]. To overcome this issue, numerous pathways were adopted, such as reducing the drug particle size [10], conversion to simple salts [11-13], formation of cleavable analogues (prodrugs) [14], the utility of surfactants [15], formation of complexes [16], suspension in lipid excipients [17], use of carrier matrix methods, etc. [18]. To improve the absorption, distribution, metabolism, and elimination (ADME) features of low water soluble drug **1,** several prodrugs were reported to enhance the therapeutic efficacy of **1** at reduced dosage [19]. An innovative approach was followed by the use of molecular orbital concepts like density functional theory (DFT), semi-empirical, and *ab-initio* for the configuration of new prodrugs [20]. Prior art search had disclosed numerous prodrugs of **1**, where the -OH group was replaced by different moieties. A carbonate prodrug **2** was reported to have better therapeutic efficacy towards PCP compared to the administration of **1** in conventional form. It was due to its better water solubility (up to 30 mg/mL) [21]. Synthesis of various kinds of derivatives was done by the reaction of **1** with substituted carbonates under the influence of phosgene and pyridine. Furthermore, they were converted to respective HCl salts and tested for their biological activity towards protozoans/parasites [22]. Twenty prodrugs **3-22** were reported disclosing the synthesis and antimicrobial studies (except for **9** & **10**). Among them, eighteen prodrugs had exhibited potent activity, which is comparable to that of **1** and much higher than Chloroquine. Various substituted halo derivatives were reacted with **1** in the presence of potassium carbonate (K_2CO_3) to get **3-8**. Triethylamine was used for the condensation on **9-11**, **18**, **19,** and **21**. Furthermore, the use of 10% sodium hydroxide gave **12-17**, **20,** and **22**. The ester analogues were found to be more potent biologically than the corresponding ether analogues [23]. Four prodrugs, **23-26,** were reported as per the computational configuration approach. The work also explores the estimation of drug **1** release rate from its respective prodrugs, which is in accordance with the nature of linked-up moiety as per Kirby's enzyme model [24]. Five prodrugs **27-31** were reported, which are configured based on the computational strategy. These prodrugs are the dicarboxylic-semi-ester analogues of **1**. This work emphasizes the nature and type of the linker moiety, which forms the limiting factor for the cleavage rate of the prodrug to its parent drug **1** [25]. Based on the positive information gained from the computational design method, prodrug **30** was prepared by the interaction of **1** with succinic anhydride [$(CH_2CO)_2O$] under the influence of sodium hydride (NaH). Hydrolysis data of **30** was gathered at various pH levels like 2.2, 5.5, 7.4, and 1N HCl. The rate of hydrolysis ($t_{1/2}$) was found to be different in each distinct medium, ranging from a few seconds to a few years. Thus, the linker-based cleavage study can be done to design the prodrugs [26]. In our previous work, **33** and **34** were coupled under silver nitrate mediation to get **35**, upon hydrolysis by KOH gave **1** [27-30]. The reaction of **36** with **1** in the presence of K_2CO_3 and acetonitrile gave the prodrug **32**, its hydrolysis resulted in the regeneration of drug **1** along with the formation of metabolite **37**. A better water solubility (3.75 µg/mL) was observed for **32** with a relatively faster rate of hydrolysis [31].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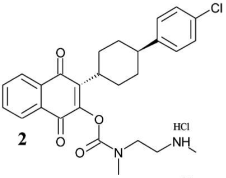

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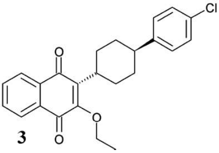
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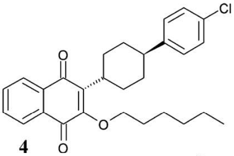
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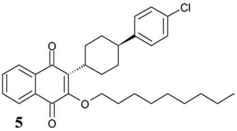
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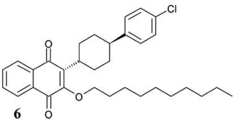
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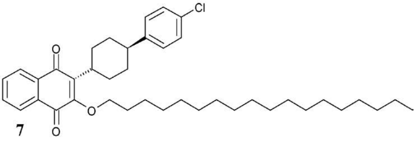
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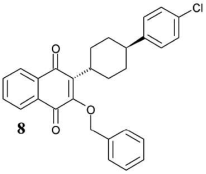
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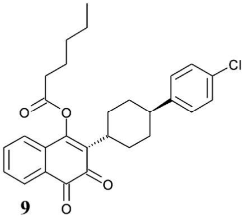
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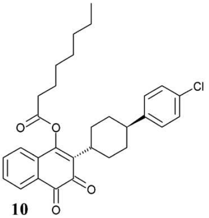
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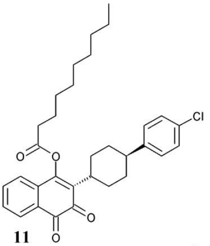
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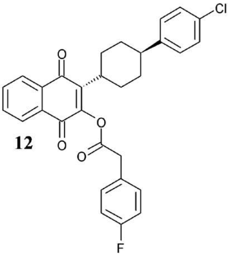
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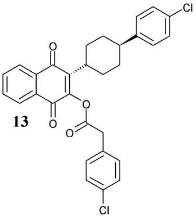
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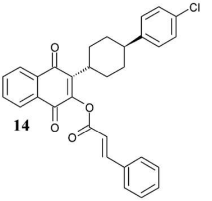
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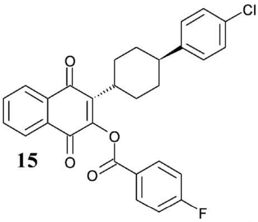

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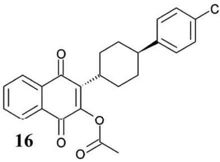

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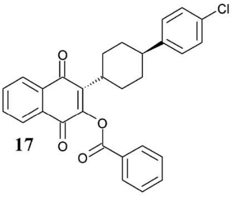

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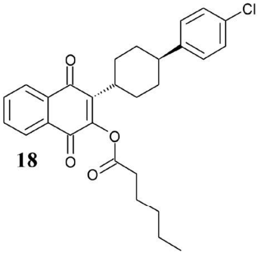

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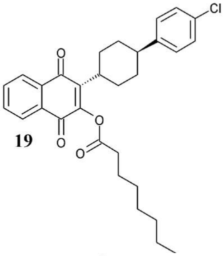

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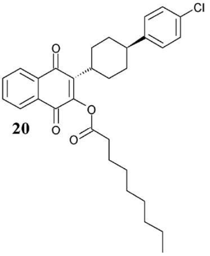

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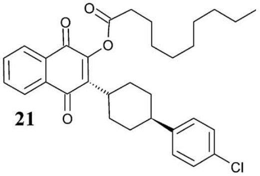

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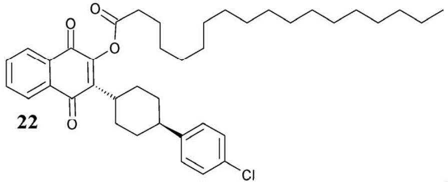

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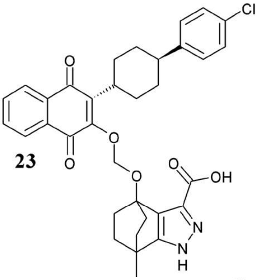

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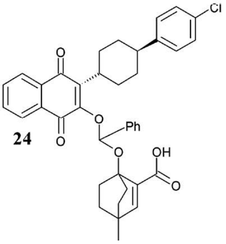

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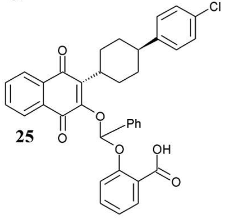

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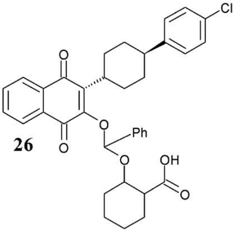

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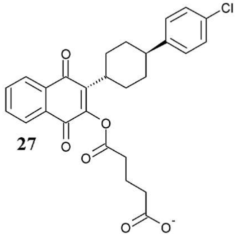

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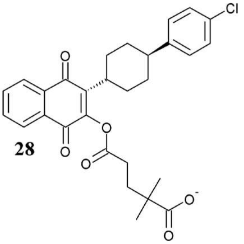

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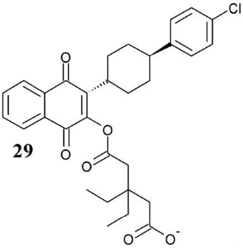
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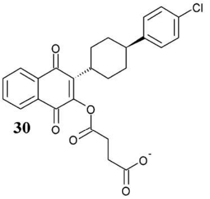
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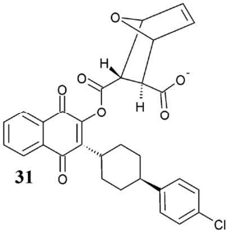
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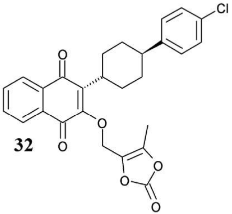
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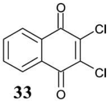
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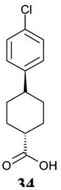
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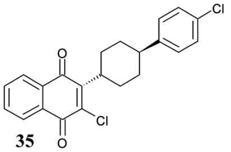
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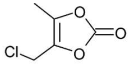
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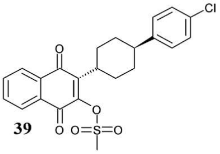
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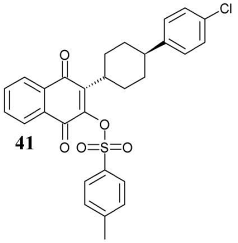
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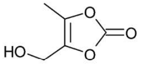
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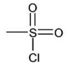
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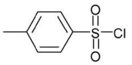
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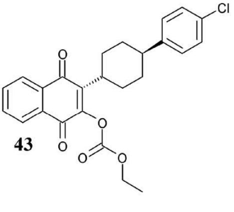
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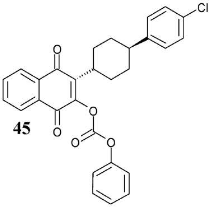
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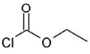
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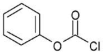
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In the present work, we report the synthesis and structural elucidation of some new sulfonate and carbonate prodrugs of **1** with better water solubility and an admirably fast hydrolysis rate. These prodrugs would enhance the therapeutic efficacy of **1** with increased bioavailability. A replicable and scalable process was designed with better yield and purity, which would assist in the commercialization of disclosed prodrugs in the future.

2. Materials and Methods

Present research work involves the strategic synthesis of four new prodrugs of **1**. Among them, two produgs were embedded with sulfonate linker, and the other prodrugs were with carbonate linkers as the bio-cleavable groups. The parent drug **1** was prepared using our own refurnished method, using **32** and **33** as the key starting materials. The reagents like methane sulfonyl chloride **38,** ethyl chloroformate **42**, phenyl chloroformate **44**, and K_2CO_3 were procured from Rankem, and 4-toluene sulfonyl chloride **40** was procured from Sd-fine chem. The solvents (acetonitrile and ethyl acetate) and concentrated hydrochloric acid were procured locally in commercial grades. Experiments were conducted using the procured reagents and solvents without any additional purification processes.

Melting points (m.p) of all four new prodrugs were noted by the open capillary pathway from the Buchi apparatus and are uncorrected. ^{1}HNMR spectra were obtained (in DMSO-d_6/$CDCl_3$) from a 400MHz NMR spectrometer (Bruker) using tetramethylsilane (TMS) as an internal reference standard. Coupling constants *J* are in Hz, and multiplicities are represented as a singlet (s), doublet (d), triplet (t), broad singlet (bs), and multiplet (m). Mass spectra were obtained from the Agilent mass spectrometer, which was operated at 70ev. Progress of the reaction, reaction completion, and product purity were gathered by thin layer chromatography (TLC) with the use of pre-coated silica/alumina TLC plates (Merck $^{60}F_{254}$) and HPLC.

2.1. General procedure for the synthesis of prodrugs

1 (5.00 g, 0.0136 mol), K_2CO_3 (4.70 g, 0.0340 mol), and 100 mL of acetonitrile were taken in a reactor provided with a guard tube having fused anhydrous silica. Through the dropping funnel, sulfonyl/ carbonate derivative [**38** (2.34 g, 0.0204 mol for **39**), **40** (3.89 g, 0.0204 mol for **41**), **42** (2.95 g, 0.0276 mol for **43**), **44** (4.26 g, 0.02726 mol for **45**)] was added to the reactor under stirring at room temperature (RT). Temperature of the reaction mixture was raised to 60-65 ^{0}C and kept at the same temperature for 2 h (for **39**), 6 h (for **43**), 8 h (for **41**) and 11 h (for **45**). TLC methodology was employed to monitor the reaction progress and completion. The solvent was removed under reduced pressure to get the sticky residue. To the crude sticky residue, add 30 mL of aqueous ethyl acetate (25 mL of ethyl acetate and 5 mL of water) and keep under stirring at RT for 1 h. The suspended solid was filtered under suction and dried it at 40-45 ^{0}C for 2 h under reduced pressure to isolate the respective analogues of **1** as prodrugs. Preliminary details of isolated prodrugs are furnished in **Table 1**.

Table 1: Preliminary data of the isolated prodrugs

C. No.	Molecular Formula (m.f)	Molecular Weight (m.w)	Melting Point (m.p)	Yield (%)
39	$C_{23}H_{21}ClO_5S$	444.92	222-225	88
41	$C_{29}H_{25}ClO_5S$	521.02	186-190	80
43	$C_{25}H_{23}ClO_5$	438.90	192-194	66
45	$C_{29}H_{23}ClO_5$	486.94	202-204	78

2.2. Characterization data of 39

2-[*trans*-4-(4-Chlorophenyl)cyclohexyl]-3-mesyloxy-1,4-naphthaquinone, ^{1}HNMR spectrum (in $CDCl_3$), 300 MHz: 8.08-8.14 (2H, m), 7.74-7.82 (2H, m), 7.26-7.28 (2H, d, *J*=6.0 Hz), 7.17-7.20 (2H, d, *J*=9.0 Hz), 3.66 (3H, s), 3.18-3.26 (1H, m), 2.64-2.72 (1H, m), 1.51-2.31 (8H, m). Mass spectrum, m/z: 365.2 (M^+-1, mesyl group cleaved).

2.3. Characterization data of 41

2-[*trans*-4-(4-Chlorophenyl) cyclohexyl]-3-tosyloxy-1,4-naphthaquinone, ^{1}HNMR spectrum (in $CDCl_3$), 300 MHz: 8.02-8.11 (4H, m), 7.71-7.79 (2H, m), 7.44-7.47 (2H, d, *J*=7.8 Hz), 7.26-7.28 (2H, d, *J*=8.1 Hz), 7.12-7.15 (2H, d, *J*=8.4 Hz), 3.01-3.09 (1H, m), 2.57-2.65 (1H, m), 2.51 (3H, s), 2.20-2.29 (2H, m), 1.41-2.17 (6H, m). Mass spectrum, m/z: 365.2 (M^+ -1, tosyl group cleaved).

2.4. Characterization data of 43

2-[*trans*-4-(4-Chlorophenyl) cyclohexyl]-1,4-dihydro-1,4-dioxonaphthalen-3-yl ethyl carbonate, ^{1}HNMR spectrum (in $CDCl_3$), 400 MHz: 8.10-8.15 (2H, m), 7.73-7.79 (2H, m), 7.27-7.29 2H, d, *J*=9.32 Hz), 7.17-7.19 (2H, d, *J*=8.24 Hz), 4.38-4.44 (2H, q, *J*=7.08 Hz), 3.13-3.19 (1H, m), 2.61-2.67 (1H, m), 1.53-2.09 (8H, m), 1.44-1.47 (3H, t, *J*=7.10 Hz). Mass spectrum, m/z: 439.1 (M^+), 438.1 (M^+ -1), 440.1 (M^+ +1), 441.2 (M^+ +2).

2.5. Characterization data of 45

2-[*trans*-4-(4-Chlorophenyl) cyclohexyl]-1,4-dihydro-1,4-dioxonaphthalen-3-yl phenyl carbonate, ^{1}HNMR spectrum (in $CDCl_3$), 400 MHz: 8.12-8.16 (2H, m), 7.73-7.79 (2H, m), 7.18-7.47 (9H, m), 3.19-3.25 1H, m), 2.64-2.71 (1H, m), 1.56-2.14 (8H, m).

2.6. Solubility estimation method

We employed the shake-flask method [32, 33] to establish the solubility profile of prodrugs. A known quantity of prodrug was added to a known volume of DM water in a clean conical glass flask and kept inside the sonicator instrument for 30 min at RT (30 ^{0}C). The mixture obtained was filtered carefully to isolate the insoluble prodrug and then dried in a vacuum oven to estimate the exact weight of the insoluble prodrug. This procedure was implemented to determine the solubility profile of all four prodrugs of the present invention in duplication, and an intended solubility profile was established.

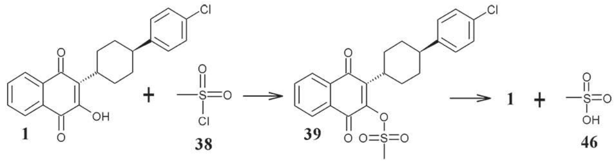
2.7. General procedure for hydrolysis

Hydrolysis was carried out by adding 1.0 g of prodrug sample to 100 mL of acidic buffer solution (aqueous HCl) having pH 2.0-2.2 and stirring at RT for 5-20 h. The progress of the reaction (cleavage) was monitored by TLC (0.5 mL of sample was diluted in 1 drop DMF to get a clear solution). In this way, the hydrolysis profile of all the four prodrugs was established.

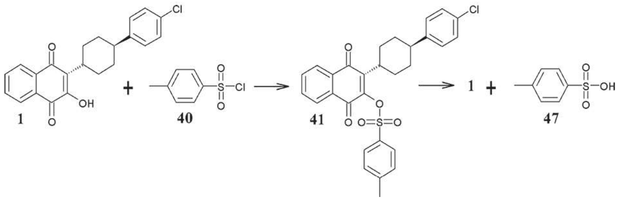
3. Results and Discussion

3.1. Chemistry

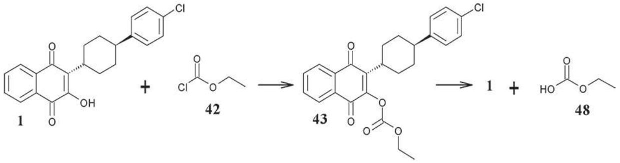
Interaction of K_2CO_3 with **1** generates the phenolate anion, this will selectively displace the highly reactive chlorine in **38** to give **39** (Scheme 1), similarly in **40** to give **41** (Scheme 2), in **42** to give **43** (Scheme 3) and in **44** to give **45** (Scheme 4) respectively. A slow conversion rate was observed in all the above experiments, which can be attributed to steric hindrance offered by **1**. Aqueous ethyl acetate slurry wash for the crude residue was incorporated specifically to eliminate the impurities and the inorganics generated during the respective reactions. The isolated yields of prodrugs are reasonably good, with better purity and reproducibility, a vital factor for prodrug commercialization in the future.



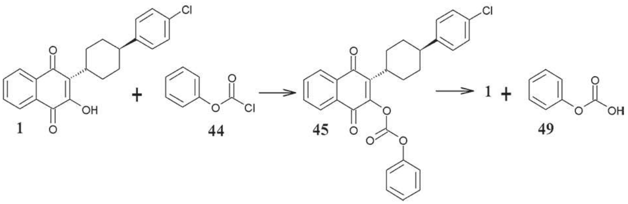
Scheme 1. Synthesis of **39** and its hydrolysis to release **46**



Scheme 2. Synthesis of **41** and its hydrolysis to release **47**



Scheme 3. Synthesis of **43** and its hydrolysis to release **48**



Scheme 4. Synthesis of **45** and its hydrolysis to release **49**

3.2. Solubility profile

From the prior art findings, we could able to observe the efforts put in to improve the bioavailability of **1** in numerous ways. Water solubility of **1** at RT was reported to be 0.74 µg/ mL [22]. We have subjected all the prodrugs of the present invention for water solubility estimation under sonication at RT. As per the study, approximate solubility was observed to be around 1.15 µg/mL for **39**, 2.47 µg/mL for **41**, 3.32 µg/mL for **43,** and 2.89 µg/mL for **45,** respectively. All four novel prodrugs of the present invention exhibited reasonably better water solubility, thus contributing to the bioavailability and clinical efficacy of **1**.

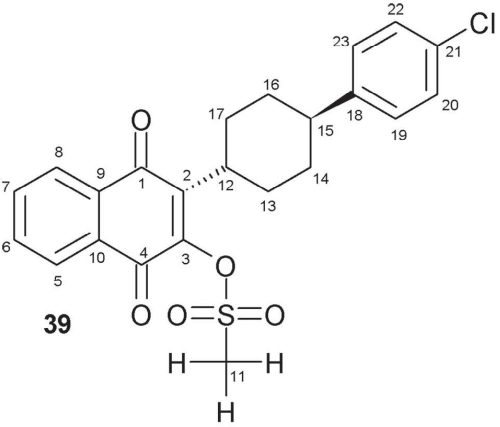
3.3. Feasibility of hydrolysis

Therapeutically active drug **1** will be released upon *in-vitro* hydrolysis of prodrugs with the release of respective metabolites. The primary metabolites released post hydrolysis of prodrugs are, **39** will release methanesulfonic acid **46**, likewise **41** releases 4-methylbenzenesulfonic acid **47**, **43** releases ethyl hydrogen carbonate **48** and **45** releases phenyl hydrogen carbonate **49** respectively along with the release of parent drug **1** (Scheme 1, 2, 3 & 4). A prodrug must release the parent drug along with non-toxic metabolites during *in-vitro* cleavage process. Preliminary hydrolysis of these prodrugs was carried out by the use of an acidic buffer solution (aqueous HCl) of pH 2.0-2.2. As per the tests conducted at RT and TLC monitoring, **39** had undergone hydrolysis in 5-6 h, similarly **41** at 7-8 h, **43** at 9-10 h, and **45** at 19-20 h, respectively. Extended hydrolysis study has to be conducted at various pH levels to get the blueprint of their pharmaco-kinetic behavior prior to *in-vivo* studies.

Furthermore, there are numerous drugs that are under clinical practice as sulfonic acid salts like (mesylate, tosylate, besylate, edisylate, esylate, closylate etc) [34, 35]. Mild reaction conditions have to be adopted during the synthesis of sulfonic acid salts to avoid the formation of genotoxic/carcinogenic short-chain alkyl esters (n=1-3) of respective sulfonic acids [36, 37]. Similarly, sulfonate linker embedded prodrugs would get bio-cleaved under the enzymatic impact and contribute to enhanced drug bioavailability [38, 39].

Likewise, there were quite a few prodrugs that have characteristic bio-cleavable linkers [40, 41]. These prodrugs have good chemical and proteolytic stability and, hence, form the structural or functional part of many therapeutic agents [42, 43].

3.4. Structural elucidation



39

The structure of **39** was elucidated based on the ^{1}HNMR and mass spectrum. The carbon atoms of the prodrug **39** were numbered for the purpose of structural elucidation. The 300 MHz ^{1}HNMR spectrum of **39** showed a multiplet in the range δ 8.08-8.14 ppm was assigned for 2 protons attached to C-5 and C-8. The multiplet in the range δ 7.74-7.82 ppm was assigned for 2 protons attached to C-6 and C-7. Aromatic protons resonated as a doublet (J=6.0 Hz) in the range δ 7.26-7.28 ppm integrated for 2 protons which are which are attached to C-20 and C-22. Aromatic protons that resonated as a doublet (J =9.0 Hz) in the range δ 7.17-7.20 ppm integrated for 2 protons, which are attached to C-19 and C-23. The singlet at δ 3.66 ppm was assigned for 3 protons attached to C-11. The multiplet in the range δ 3.18-3.26 ppm was assigned for one proton attached to C-12. The multiplet in the range δ 2.64-2.72 ppm was corresponding to a proton attached to C-15. The remaining 8 protons attached to C-13, C-14, C-16, and C-17, respectively, were all resonated as multiplets in the range δ 1.51-2.31 ppm. The mass spectrum of **39** showed a molecular ion peak (mesyl group cleaved) with m/z values of 365.2 (M^{+} -1) with a molecular weight of 444.92 (mesyl group intact).

Supplementary Information (SI): Associated spectral details of isolated prodrugs were provided in the SI file (**39**, Figure S1 to S3, for **41**, Figure S4 to S6, for **43**, Figure S7 to S9 and for **45**, Figure S10 to S11b).

Conclusion

In this work, an intended molecular modification to **1** was achieved by the synthesis of four novel prodrugs. This initiative was primarily aimed at overcoming the solubility issues associated with **1**. Among the four, two prodrugs of **1** have sulfonate linkers, and the other two have carbonate linkers in their molecular framework. These linkers are renowned for hydrolyzing easily in biological/enzymatic conditions to release the parent drug moiety and hence contribute to an increase in the drug bioavailability. Furthermore, a replicable synthetic process to isolate prodrugs with good yield and purity was provided for future scalability. This rational modification strategy was done to refurnish the physico-chemical and pharmacological features of **1**, specifically to improve its water solubility and pharmaco-kinetic behavior. The higher water solubility profile of these prodrugs will increase the bioavailability and clinical efficacy of **1**. Further studies with regard to animal-based administering studies and *in-vivo* pharmaco-kinetics, can push them as the formidable future prodrugs for routine clinical practice of **1** at a reduced dosage.

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Supplementary Information (SI)

The spectral details which are supportive to structural elucidation of novel prodrugs of **1** are available in the SI file (**Figure S1** to **S11b**, page no. 3 to 10).

Author contributions

Sanjay; had collected the prior arts, performed experiments, analyzed the result outputs, and wrote the entire manuscript. *Shashiprabha*; had assisted to design experiments and helped for the spectral analysis (molecular structure interpretations). *Shridhara*; had monitored the entire work flow, manuscript correction and anchored the entire research initiative.

Conflict of Interest

The authors hereby declare no potential conflicts of interest with respect to the research, funding, authorship, and/or publication of this article

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