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Design, synthesis and evaluation of diclofenac-antioxidant mutual prodrugs as safer NSAIDs

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Diclofenac has been conjugated with different antioxidants having antiulcerogenic activity with the objective of obtaining diclofenac-antioxidant mutual prodrugs as safer NSAIDs devoid of ulcerogenic side-effects. The synthesized derivatives are screened for their antiinflammatory, analgesic and antiulcer activity. The mutual prodrugs show retention of antiinflammatory activity with reduced ulcerogenic side-effects. These results indicate that diclofenac-antioxidant mutual prodrugs have the potential to be developed as safer NSAIDs.

Keywords: NSAIDs, diclofenac, mutual prodrug, antioxidants, ulcerogenicity

Nonsteroidal antiinflammatory drugs (NSAIDs) are the most widely used drugs, with prescription as well as over the counter formulations being available in most countries. Since the introduction of NSAIDs in the market, enormous literature has been published regarding their side-effects. Although these agents affect renal and cardiovascular systems, the most common, widely studied, reported and reviewed side-effects are related to gastrointestinal tract (GIT)[1-4]. The pharmacological activity of NSAIDs is related to their ability to inhibit the activity of the enzyme cyclo-oxygenases (COXs) involved in the biosynthesis of prostaglandin H_2 (PGH_2)[5,6]. It is now well known that COX exists in two isoforms, namely COX-I and COX-II, which are regulated differently. COX-I is constitutively expressed in stomach to provide cytoprotection in the GIT. COX-II is inducible and plays a major role in prostaglandin biosynthesis in inflammatory cells[7]. Since most of the NSAIDs used clinically inhibit both isoforms, long term use of these agents results in gastric ulcer and there is enough evidence that inhibition of COX-I rather than that of COX-II underlies gastric ulcer formation[8-12]. As a result, a number of selective COX-II inhibitors, including Celecoxib and Rofecoxib have been introduced for clinical use with exceptional antiinflammatory properties and reduced gastric toxicity[13]. But initial enthusiasm for selective COX-II inhibitors as safer NSAIDs has faded due to emergence of serious cardiovascular side-effects on long term use[11].

It has been well known that local generation of various reactive oxygen species (ROS) plays a significant role in the formation of gastric ulceration associated with NSAID therapy[14-17]. These observations indicate that antioxidants may be used to prevent NSAIDs induced gastric ulcers. During the past few decades, a large number of naturally occurring compounds have been identified as antioxidants, which are viewed as promising therapeutic agents for treating free radical mediated diseases including NSAID induced peptic ulcers. Large number of herbs and spices are recognized as source of natural antioxidants and studies have confirmed their efficacy for the treatment of gastrointestinal ulcers[18]. Based on these observations, it has been suggested that coadminstration of antioxidants and NSAIDs in formulated dosage form may possibly decrease the risk of NSAIDs induced gastrointestinal side-effects[19,20]. However, there are potential advantages in giving such coadministered drugs having complementary pharmacological activities in the form of a single chemical entity. Such agents are named as mutual prodrugs which are designed with improved physicochemical properties and release the parent drug at the site of action[21,22]. In this paper we report the synthesis and evaluation of diclofenac-antioxidant mutual prodrugs as safer NSAIDs. Study on physicochemical properties to assess their prodrug potential is underway and will be subject of a separate report.

Results and Discussion

Prodrug may be defined as pharmacologically inactive chemical derivative of a parent drug molecule requiring chemical and/or enzymatic transformation within the body to release the parent drug[23]. Most commonly used prodrugs are ester derivatives of carboxyl and hydroxyl groups containing drug molecules. The popularity of using esters as a prodrug type for drugs containing carboxyl or hydroxyl functions stems primarily from the fact that the esterases present in the body are capable of hydrolyzing esters. The distribution of esterases is ubiquitous and several types can be found in blood, liver and other organs and tissues[24]. Sometimes, simple aliphatic or aromatic esters may not be sufficiently labile *in vivo* to ensure a sufficiently high rate and extent of prodrug reconversion to the parent drug. This shortcoming can be overcome by preparing double ester type, in which the terminal ester group is less sterically hindered. In the present study, the mutual prodrugs **5a-g** were synthesized using glycolic acid spacer (-OCH_2COO-) and evaluated for pharmacological activity.

Synthesis

The mutual prodrugs **5a-g** were synthesized using glycolic acid spacer (-OCH_2COO-). For the preparation of diclofenac-antioxidant mutual prodrugs, various natural antioxidants were identified for conjugation including, guaiacol **1a**, eugenol **1b**, thymol **1c**, vanillin **1d**, sesamol **1e**, umbelliferone **1f** and menthol **1g**. These agents have been an important part of human diet and therefore their safety profile is well known[25-28]. Chloroacetyl chloride **2** was reacted with different antioxidants **1a-g** in the presence of triethylamine using dichloromethane as solvent to obtain the required chloroacetyl derivatives **3a-g**. These chloroacetyl derivatives were condensed with diclofenac **4** in the presence of triethylamine and sodium iodide using DMF as solvent. The sequence of steps of these reactions is shown in **Figure 1**. The resulting mutual prodrugs **5a-g** were obtained in reasonable yield (43-58%). The structures of all synthesized compounds were confirmed using of elemental analysis and spectral studies **(Table I)**.

Spectral studies

The IR spectra of the prodrugs showed absorption peaks at around 3360 cm^{-1} for N-H stretching and at around 1760 cm^{-1} characteristic of C=O. In ^1H NMR, the signals for methylene protons (Ar-CH_2) of the parent structure were observed in the range of δ 3.5 to δ 4.02. Additionally, it showed a signal at around δ 4.92 to 5.01 for –OCH_2. Signals for the protons of the promoieties also appeared in the aromatic **5a-f** and aliphatic region **5g** respectively. In ^{13}C NMR spectra, the signals for aromatic carbons had a spread from δ 103 to 150. Signals for other carbons of the parent structures were observed at about δ 38 (Ar-CH_2) and 170 (*C*OO). Additional peaks for OCH_2 and *C*OO were observed at δ 60 and δ 165 respectively.

Pharmacological Evaluation

The parent drug has been used as reference substance. Antiinflammatory activity was determined by using carrageenan induced rat paw edema model. Carrageenan (1% w/v) was used to produce paw edema. Edema is presented as percentage increase in right hind paw, in comparison to the uninjected left hind paw. Percentage change in paw volume was calculated and expressed as the amount of inflammation[29]. For antiinflammatory activity, the test compounds **5a-g** were administered orally at molar equivalent doses of diclofenac (10 mg/kg, p.o.). All these derivatives at molar equivalent doses significantly reversed carrageenan-induced inflammation. Diclofenac-eugenol **5b** and diclofenac-sesamol **5f** conjugates showed increased antiinflammatory activity than that of diclofenac. This increased activity may be due to the contribution by their promoieties. Furthermore, equimolar mixtures of diclofenac and promoieties were also studied for the antiinflammatory activity. These physical mixtures showed comparable results to the parent diclofenac, but lower than their corresponding conjugates (**Table II**). These observations indicate that there is an added advantage in combining these agents with antioxidant promoieties having complementary pharmacological activities in the form of a single molecule, i.e. prodrug, resulting in improved physicochemical properties.

For the analgesic activity, abdominal writhing assay was performed[30]. Writhing was induced by intraperitoneal (i.p.) injection of freshly prepared acetic acid solution (1%, 10 mL/kg, i.p.) in mice. The number of writhes (constriction of abdomen, turning of trunk, and extension of hind limbs) due to acetic acid was expressed as a nociceptive response. Vehicle treated control mice were given 1% acetic acid and writhing response was noted for 20 min. Diclofenac

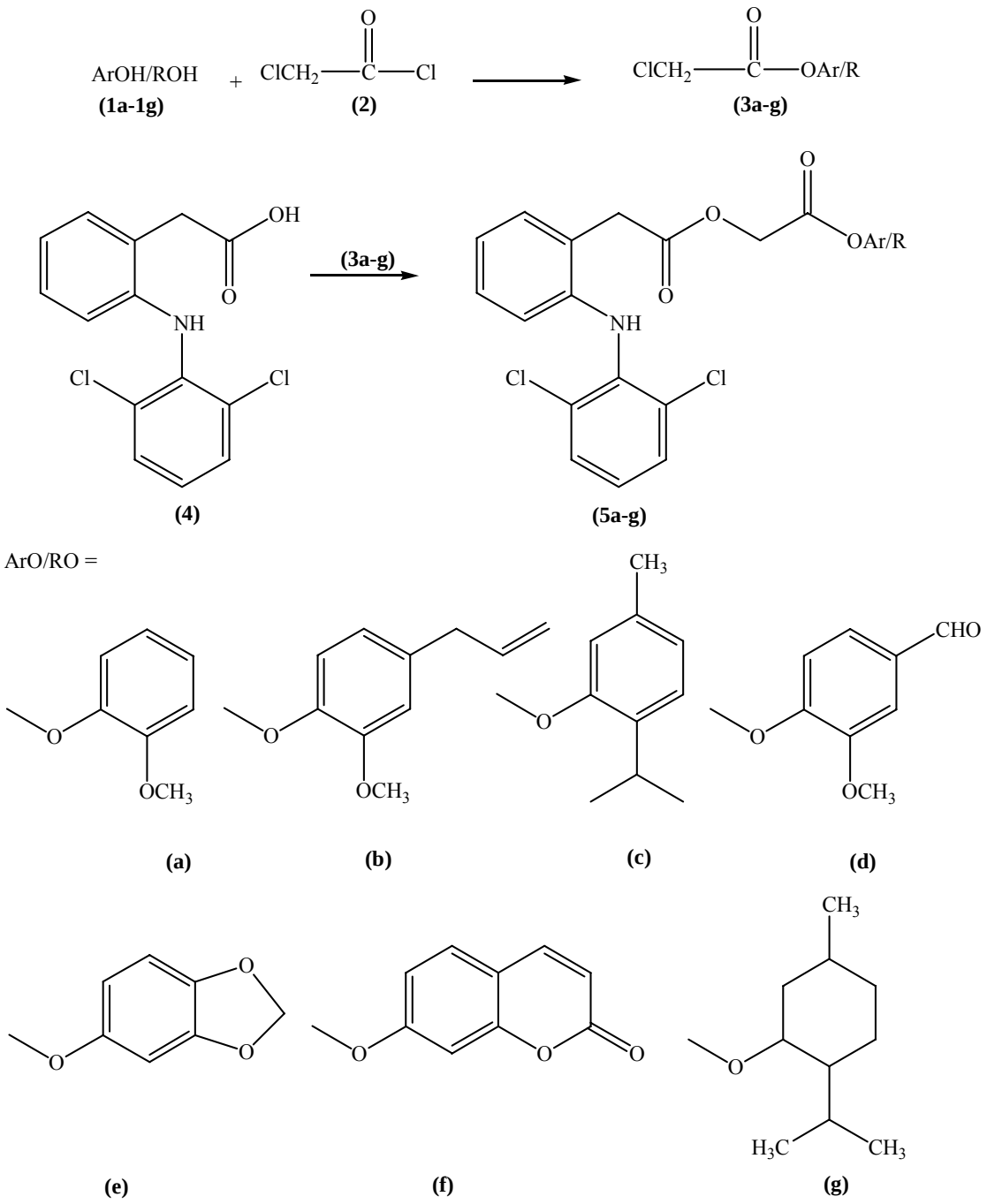

Figure 1 — Sequence of steps involved in the synthesis of diclofenac-antioxidant mutual prodrugs with OCH_2COO- spacer

(10 mg/kg, p.o.) as well as synthesized mutual prodrugs at molar equivalent doses significantly reduced the writhing response **(Table II)**. The results showed that these derivatives **5a-g** exhibited retention of analgesic activity.

The prodrugs were screened for their ulcerogenicity in rats, using parent drug induced acute gastric ulcerations[31]. The animals were fasted for 24 h, divided into different groups containing 6 animals in each group. Control group was treated with an equal volume of 0.5% carboxymethylcellulose (CMC) vehicle. Animals were sacrificed 8 hr after the treatment. The stomach was removed, opened along the greater curvature, washed with saline, and

Table I — Physical properties, spectral and elemental data of antioxidant chloroacetyl derivatives and diclofenac-antioxidant mutual prodrugs

Compd	Yield (%)	m.p. (ºC)	Spectral and Elemental data
2-Methoxyphenyl chloroethanoate **(3a)** $C_9H_9ClO_3$	80.5	45-47	The mp, ^{1}H NMR and ^{13}C NMR data of **(3a)** were consistent with those previously reported.[28] IR (KBr): 3050.3, 2947.0 (C-H), 1781.5 (C=O), 1142.4 (C-O), 751.6 (C-Cl) cm^{-1}.
2-Methoxy-4-(2-propenyl)phenyl chloroethanoate **(3b)** $C_{12}H_{13}ClO_3$	75.4	Semisolid	^{1}H NMR and ^{13}C NMR data of **(3b)** were consistent with those previously reported.[28] IR (KBr): 3075.5, 2962.1 (C-H), 1778.7 (C=O), 1145.0 (C-O), 750.5 (C-Cl) cm^{-1}.
2-Isopropyl-5-methylphenyl chloroethanoate **(3c)** $C_{12}H_{15}ClO_2$	86.2	Semisolid	^{1}H NMR and ^{13}C NMR data of **(3c)** were consistent with those previously reported.[28] IR (KBr): 3025.7, 2965.1 (C-H), 1775.5 (C=O), 1153.3 (C-O), 817.0 (C-Cl) cm^{-1}.
4-Formyl-2-methoxyphenyl chloroethanoate **(3d)** $C_{10}H_9ClO_4$	79.8	82-84	IR (KBr): 3035.2, 2956.7 (C-H), 1766.1 (C=O), 1149.9 (C-O), 814.0 (C-Cl) cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 3.93 (s, 3H, OC*H*$_3$), 4.39 (s, 2H, -C*H*$_2$), 7.27-7.29 (m, 1H, Ar-*H*), 7.50-7.53 (m, 2H, Ar-*H*), 9.98 (s, 1H, C*H*O); ^{13}C NMR ($CDCl_3$): δ 40.52 ($ClCH_2$), 56.19 (OCH_3), 110.98-144.22 (Ar-carbons), 151.68 (Ar*C*-OCH_3), 164.89 (*C*=O), 190.96 (*C*HO); Anal. Calc. for $C_{10}H_9ClO_4$: C, 52.53; H, 3.97. Found: C, 52.64; H, 3.99%.
3,4-(Methylenedioxy)phenyl chloroethanoate **(3e)** $C_9H_7ClO_4$	85.7	46-48	IR (KBr): 3033.9, 2941.8 (C-H), 1767.3(C=O), 1154.3(C-O) , 801.5 (C-Cl) cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 4.30 (s, 2H, -C*H*$_2$), 6.02 (s, 2H, OC*H*$_2$O), 6.59 (dd, 1H, *J* = 2.4 Hz and 8.4 Hz, Ar-*H*), 6.66 (d, 1H, *J* = 2.4 Hz, Ar-*H*), 6.81 (d, 1H, *J* = 8.4Hz, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 40.83 ($ClCH_2$), 101.86 (OCH_2O), 103.30-148.13 (Ar-carbons), 166.21 (*C*=O); Anal. Calc. for $C_9H_7ClO_4$: C, 50.37; H, 3.29. Found: C, 50.45; H, 3.43%.
2-Oxo-2*H*-chromen-7-yl chloroethanoate **(3f)** $C_{11}H_7ClO_4$	83.4	160-162	IR (KBr): 3023.7, 2946.3 (C-H), 1790.7 (C=O), 1735.5 (C=O),1165.7 (C-O), 836.7 (C-Cl) cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 4.35 (s, 2H, -C*H*$_2$), 6.42 (d, 1H, *J* = 9.6, Ar-*H*), 7.10 (dd ,1H, *J* = 8.5 Hz and 2.2 Hz, Ar-*H*), 7.17 (d, 1H, *J* = 2.2 Hz, Ar-*H*), 7.53 (d, 1H, *J* = 8.5 Hz, Ar-*H*), 7.71 (d, 1H, *J* = 9.6 Hz, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 40.80 (Ar-*C*H$_2$), 110.23 (Ar-CH=*C*H-), 116.60-154.5973 (Ar-carbons), 142.77 (Ar-*C*H=CH-), 160.20 (*C*=O, umbelliferone), 165.37 (*C*=O); Anal. Calc. for $C_{11}H_7ClO_4$: C, 55.37; H, 2.96. Found: C, 55.45; H, 3.19%.
2-Isopropyl-5-methylcyclohexyl chloroethanoate **(3g)** $C_{12}H_{21}ClO_2$	80.8	35-37	^{1}H NMR and ^{13}C NMR data of **(3g)** were consistent with those previously reported.[28] IR (KBr): 2961.0 (C-H), 1750.0 (C=O), 1196.2 (C-O), 788.3 (C-Cl) cm^{-1}.
2-Methoxy phenyl-2-[2(2,6-dichloro phenylamino)phenyl]ethanoyloxy ethanoate **(5a)** $C_{23}H_{19}Cl_2NO_5$	50.2	110-112	IR (KBr): 3368.8 (N-H), 3063.1 and 2949.0 (C-H), 1760.4 (C=O), 1604 (C=C), 1262.6 (C-O) cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 3.81 (s, 3H, OC*H*$_3$), 3.97 (s, 2H, Ar-C*H*$_2$), 4.97 (s, 2H, OC*H*$_2$) 6.56 (d, 1H, *J* = 8.0 Hz, Ar-*H*, diclofenac), 6.74 (s, 1H, NH, D_2O exchangeable), 6.90-6.99 (m, 4H, Ar-*H*, diclofenac, guaiacol), 7.03-7.04 (dd, 1H, *J*=1.66 Hz and 7.9 Hz, Ar-*H*, guaiacol), 7.11-7.15 (m, 1H, Ar-*H*, diclofenac),7.18-7.23 (m, 1H, Ar-*H*, guaiacol), 7.28 (dd, 1H, *J*=1.4 Hz and 8.4 Hz, Ar-*H*, diclofenac), 7.33 (d, 2H, *J* =8.0 Hz, Ar-*H*, diclofenac); ^{13}C NMR ($CDCl_3$): δ 38.05 (Ar-*C*H$_2$), 55.87 (OCH_3), 60.99 (OCH_2COO), 112.45-142.78 (Ar-carbons), 150.86 (Ar*C*-OCH_3), 165.73 (CH_2COO), 171.41 (Ar-CH_2COO). LC-MS *m/z* 460.06 $[M]^+$; Anal. Calc. for $C_{23}H_{19}Cl_2NO_5$: C, 60.01; H, 4.16; N, 3.04. Found: C, 60.11; H, 4.32; N, 3.08%.

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Table I — Physical properties, spectral and elemental data of antioxidant chloroacetyl derivatives and diclofenac-antioxidant mutual prodrugs — *Contd*

Compd	Yield (%)	m.p. (ºC)	Spectral and Elemental data
2-Methoxy-4-(2-propenyl)phenyl-2-[2(2,6-dichloro phenylamino)phenyl] ethanoyloxy ethanoate **(5b)** $C_{26}H_{23}Cl_2NO_5$	49.2	72-73	IR (KBr): 3363.8 (N-H), 3066.5 and 2964.7 (C-H), 1763.9 (C=O), 1601.8 (C=C), 1271.1 (C-O) cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 3.36 (d, 2H, *J* = 6.7 Hz, -C*H*$_2$-, eugenol), 3.79 (s, 3H, OC*H*$_3$) 3.96 (s, 2H, Ar-C*H*$_2$), 4. 96 (s, 2H, OC*H*$_2$), 5.06-5.12 (m, 2H, =C*H*$_2$, eugenol) 5.90-5.97 (m, 1H, -C*H*=, eugenol), 6.55 (d , 1H, *J* = 7.96 Hz, Ar-*H*, diclofenac), 6.73-6-77 (m, 3H, N*H*, D_2O exchangeable, Ar-*H*, diclofenac), 6.94-6.99 (m, 3H, Ar-*H*, eugenol), 7.10-7.14 (m, 1H, Ar-*H*, diclofenac), 7.25 (dd, 1H, *J* = 1.4 Hz and 7.0 Hz, Ar-*H*, diclofenac), 7.32 (d, 2H, *J* = 8.0 Hz, Ar-*H*, diclofenac) ; ^{13}C NMR ($CDCl_3$): δ 38.05 (Ar-*C*H$_2$), 40.10 (*C*H$_2$-CH=CH$_2$), 55.85 (O*C*H$_3$), 61.01 (O*C*H$_2$COO), 112.76 (CH=*C*H$_2$), 116.28 - 149.79 (Ar-carbons), 136.97 (*C*H=CH$_2$), 150.63 (Ar*C*-OCH$_3$), 165.86 (CH$_2$*C*OO), 171.42 (Ar-CH$_2$*C*OO). LC-MS *m/z* 500.11 $[M]^+$; Anal. Calc. for $C_{26}H_{23}Cl_2NO_5$: C, 62.41; H, 4.63; N, 2.80. Found: C, 62.34; H, 4.65; N, 2.95%.
2-Isopropyl-5-methylphenyl-2-[2(2,6-dichlorophenylamino)phenyl] ethanoyloxy ethanoate **(5c)** $C_{26}H_{25}Cl_2NO_4$	43.5	63-65	IR (KBr): 3364.0 (N-H), 3043.2, 2954.9 (C-H), 1761.0 (C=O), 1581.0 (C=C), 1285.5 (C-O) cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 1.15 (d, 6H, *J* = 6.9 Hz, 2C*H*$_3$), 2.29 (s, 3H, Ar-C*H*$_3$), 2.93 (sept, 1H, *J* = 6.9 Hz, C*H*), 3.97 (s, 2H, Ar-C*H*$_2$), 4.93 (s, 2H, OC*H*$_2$) 6.55 (d, 1H, *J* = 8.0 Hz, Ar-*H*, diclofenac), 6.74 (s, 1H, N*H*, D_2O exchangeable), 6.81 (s, 1H, Ar-*H*, thymol), 6.9-7.0 (m, 2H, Ar-*H*, diclofenac), 7.02 (d, 1H, *J* = 7.9 Hz, Ar-*H*, thymol), 7.11-7.15 (m, 1H, Ar-*H*, diclofenac), 7.18 (d, 1H, *J*=7.9 Hz, Ar-*H*, thymol), 7.27 (dd, 1H, *J* = 1.4 Hz and 6.1 Hz, Ar-*H*, diclofenac),7.33 (d, 2H, *J* = 8.0Hz, Ar-*H*, diclofenac) ; ^{13}C NMR ($CDCl_3$): δ 20.83 (Ar-*C*H$_3$), 23.06, (CH(*C*H$_3$)$_2$), 27.05 (*C*H(CH$_3$)$_2$), 38.04 (Ar-*C*H$_2$), 61.27 (O*C*H$_2$COO), 118.52-147.20 (Ar-carbons), 166.44 (CH$_2$*C*OO), 171.61 (Ar-CH$_2$*C*OO); LC-MS *m/z* 486.14 $[M]^+$; Anal. Calc. for $C_{26}H_{25}Cl_2NO_4$: C, 64.20; H, 5.18; N, 2.88. Found: C, 64.45; H, 5.23; N, 2.69%.
4-Formyl-2-methoxyphenyl-2-[2(2,6-dichlorophenylamino)phenyl] ethanoyloxy ethanoate **(5d)** $C_{24}H_{19}Cl_2NO_6$	58.9	68-70	IR (KBr): 3365.7 (N-H), 3064.7, 2943.8 (C-H), 1779.4 (C=O), 1752.4 (C=O), 1597.6 (C=C), 1275.6 (C-O) cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 3.91 (s, 3H, OC*H*$_3$), 4.00 (s, 2H, Ar-C*H*$_2$), 5.01(s, 2H, OC*H*$_2$), 6.58 (d, 1H, *J* =8.0 Hz, Ar-*H*, diclofenac), 6.72 (s, 1H, N*H*, D_2O exchangeable), 6.96-7.02 (m, 2H, Ar-*H*, diclofenac) 7.13-7.17 (m, 1H, Ar-*H*, diclofenac) 7.23-7.30 (m, 2H, Ar-*H*, diclofenac, vanillin), 7.35 (d, 2H, *J* = 8.04 Hz, Ar-*H*, diclofenac), 7.47-7.50 (m, 2H, Ar-*H*, vanillin), 9.97 (s, 1H, C*H*O) ; ^{13}C NMR ($CDCl_3$): δ 37.99(Ar-*C*H$_2$), 56.15 (OCH$_3$), 60.85 (O*C*H$_2$COO), 110.85-144.02 (Ar-carbons), 151.71 (Ar*C*- OCH$_3$), 165.13 (CH$_2$*C*OO), 171.40 (Ar-CH$_2$*C*OO),190.99 (*C*HO). LC-MS *m/z* 488.07 $[M]^+$; Anal. Calc. for $C_{24}H_{19}Cl_2NO_6$: C, 59.03; H, 3.92; N, 2.87. Found: C, 59.18; H, 3.99; N, 2.78%. Contd
3,4-(Methylenedioxy)-2-[2(2,6-dichloro phenylamino)phenyl]ethanoyloxy ethanoate **(5e)** $C_{23}H_{17}Cl_2NO_6$	53.4	80-82	IR (KBr): 3367.4 (N-H), 3051.2, 2942.7 (C-H) 1753.0 (C=O), 1584.0 (C=C), 1170.7 (C-O) cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 4.02 (s, 2H, Ar-C*H*$_2$), 4.92 (s, 2H, OC*H*$_2$), 5.98 (s, 2H, OC*H*$_2$), 6.56 (dd, 1H, *J* = 2.4 Hz and 8.4 Hz, Ar-*H*, sesamol), 6.62 (d, 1H, *J* = 8.0 Hz, Ar-*H*, diclofenac), 6.64 (d, 1H, *J* = 2.3 Hz , Ar-*H*, sesamol), 6.78 (d, 2H, *J* = 8.4 Hz, N*H*, D_2O exchangeable, Ar-H, sesamol) , 6.99-7.03 (m, 2H, Ar-*H*, diclofenac), 7.16-7.20 (m, 1H, Ar-*H*, diclofenac), 7.32 (dd, 1H, *J*=1.4 Hz and 7.5 Hz, Ar-*H*, diclofenac), 7.37 (d, 2H, *J* =8.0, Ar-*H*, diclofenac) ; ^{13}C NMR ($CDCl_3$): δ 38.02 (Ar-*C*H$_2$), 61.24 (O*C*H$_2$COO), 101.86 (OCH$_2$O), 103.43-148.07 (Ar-carbons), 166.43 (CH$_2$*C*OO), 171.56 (Ar-CH$_2$*C*OO). LC-MS *m/z* 474.03$[M]^+$; Anal. Calc. for $C_{23}H_{17}Cl_2NO_6$: C, 58.24; H, 3.61; N, 2.95. Found: C, 58.44; H, 3.75; N, 2.75%.

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Table I — Physical properties, spectral and elemental data of antioxidant chloroacetyl derivatives and diclofenac-antioxidant mutual prodrugs **—** *Contd*

Compd	Yield (%)	m.p. (ºC)	Spectral and Elemental data
2-Oxo-2*H*-chromen-7-yl-2-[2(2,6-dichlorophenylamino)phenyl] ethanoyloxy ethanoate **(5f)** $C_{25}H_{17}Cl_2NO_6$	55.8	139-140	IR (KBr): 3351.8 (N-H), 3051.2, 2952.4 (C-H), 1776.0 (C=O), 1733.2 (C=O), 1619.7 (C=C), 1269.6 (C-O) cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 3.98 (s, 2H, Ar-C*H*$_2$), 4.93 (s, 2H, OC*H*$_2$), 6.40 (d, 1H, *J* = 9. 6 Hz, Ar-*H*, umbelliferone), 6.56 (d, 1H, *J*=8.0 Hz, Ar-*H***,** diclofenac), 6.65 (s, 1H, N*H*, D_2O exchangeable), 6.95-7.03 (m, 3H, Ar-*H*, diclofenac, umbelliferone) 7.09 (d, 1H, *J* = 2.2 Hz, Ar-*H*, umbelliferone), 7.11-7.14 (m, 1H, Ar-*H*, diclofenac), 7.27 (dd, 1H, *J*=1.4 Hz and 7.5 Hz, Ar-*H*, diclofenac), 7.33 (d, 2H, *J* =8.0 Hz , Ar-*H*, diclofenac), 7.46 (d, 1H, *J* =8.5 Hz, Ar-*H*, umbelliferone), 7.67 (d, 1H, *J* =9.6 Hz, Ar-*H*, umbelliferone) ; ^{13}C NMR ($CDCl_3$): δ 37.96 (Ar-*C*H$_2$), 61.00 (O*C*H$_2$COO), 110.14 (Ar-CH=*C*H-), 116.40-154.59 (Ar-carbons), 142.75 (Ar-*C*H=CH-), 160.20 (*C*=O, umbelliferone), 165.52 (CH$_2$*C*OO), 171.52 (Ar-CH$_2$*C*OO). LC-MS *m/z* 498.04[M]$^+$; Anal. Calc. for $C_{25}H_{17}Cl_2NO_6$: C, 60.26; H, 3.44; N, 2.81. Found: C, 60.40; H, 3.58; N, 2.67%.
2-Isopropyl-5-methyl-cyclohexyl-2-[2(2,6-dichlorophenylamino) phenyl]ethanoyloxy ethanoate **(5g)** $C_{26}H_{31}Cl_2NO_4$	45.2	68-70	IR (KBr): 3369.6 (N-H), 3048.7, 2954.4 (C-H), 1742.9 (C=O), 1581.5 (C=C), 1212.8 (C-O) cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 0.73 (d, 3H, *J* = 7.0 Hz, C*H*$_3$), 0.77-0.91 (m, 8H, 2C*H*$_3$, 2C*H*), 0.96-1.05 (m, 1H, C*H*), 1.20-1.28 (m, 1H, C*H*), 1.41-1.46 (m, 1H, C*H*), 1.58-1.66 (m, 2H, 2C*H*), 1.77-1.81 (m, 1H, C*H*), 1.92-1.97 (m, 1H, C*H*), 3.92 (s, 1H, Ar-C*H*$_2$), 4.65 (s, 1H, OC*H*$_2$), 4.70-4.77 (m, 1H, C*H*), 6.55 (d, 1H, *J* = 8.0 Hz, Ar-*H*, diclofenac), 6.79 (s, 1H, N*H*, D_2O exchangeable), 6.95-7.00 (m, 2H, Ar-*H*, diclofenac), 7.11-7.15 (m, 1H, Ar-*H*, diclofenac), 7.25 (dd, 1H, *J* = 1.4 and 7.5 Hz, Ar-*H*, diclofenac), 7.33 (d, 2H, *J* = 8.0 Hz, Ar-*H*, diclofenac) ; ^{13}C NMR ($CDCl_3$): δ 16.31 (*C*H$_3$), 20.76 (CH(*C*H$_3$)$_2$), 22.00 (CH(*C*H$_3$)$_2$), 23.41 (*C*H$_2$), 26.25 (*C*H), 31.42 (*C*H), 34.14 (*C*H$_2$), 38.21 (Ar-*C*H$_2$), 40.61 (*C*H$_2$), 46.82 (*C*H), 61.53 (Ar-*C*H$_2$), 75.86 (*C*H), 118.55-142.89 (Ar-carbons), 167.16 (CH$_2$*C*OO), 171.52 (Ar-CH$_2$COO); LC-MS *m/z* 492.12[M]$^+$; Anal. Calc. for $C_{26}H_{31}Cl_2NO_4$: C, 63.42; H, 6.35; N, 2.84. Found: C, 63.21; H, 6.46; N, 2.96%.

observed for ulcers. For the acute gastric damage evaluation, the parent drug diclofenac was used to produce gastric ulcers. For this purpose, diclofenac (75 mg/kg, p.o.) was administered which produced a significant increase in ulcer index as compared to the control group. All the test compounds **5a-g** significantly reversed the ulcer index. Although administration of physical mixtures of diclofenac and antioxidants produced a decrease in ulcer index as compared to parent drug, their antiulcer activity was negligible as compared with that of the corresponding conjugates. This may be due to the polar nature of the antioxidants that leads to instability and poor bioavailability of the antioxidant. The results obtained in this study indicate that there is definite advantage in conjugating these antioxidant promoieties with the parent NSAID, diclofenac (**Table II**). This may be due to the combined effect of masking of carboxyl group and improved physicochemical properties of synthesized diclofenac-antioxidant conjugate, in addition to the contribution of the antioxidant.

Experimental Section

General procedure. ^{1}H NMR and ^{13}C NMR spectra were recorded using 400 MHz Bruker AC 30 NMR spectrometer (Bruker, Switzerland), using $CDCl_3$ or DMSO-d_6 as solvents with tetramethylsilane (TMS) as an internal standard at Regional Sophisticated Instrumentation Centre, Panjab University, Chandigarh. IR spectra were measured on a Perkin Elmer RX-1 spectrometer (Perkin-Elmer, Switzerland). Melting points were determined on Boetius stage apparatus and are uncorrected. Mass spectra were performed on LC Waters Allianz 2695, Mass spectrometer with MS detector ESI, Software MassLynx 4.0 (Waters, USA) at 70eV using electron ionization (EI) source. Elemental analyses were carried out on a Perkin-Elmer 2400. All solvents were freshly distilled and dried prior to use according to standard procedures[32].

General procedure for synthesis of antioxidant chloroacetyl derivatives (3a-g). A mixture of an appropriate antioxidant (0.01 mole), TEA (0.01 mole)

Table II — Antiinflammatory, analgesic and antiulcer activity of diclofenac, diclofenac-antioxidant mutual prodrugs and diclofenac + antioxidant physical mixtures.

Compd	Antiinflammatory activity			Analgesic activity		Antiulcer activity	
	Dose (mg/kg, p.o.)	% Increase in paw volume mean±SEM 2h	4h	Dose (mg/kg, p.o)	% Inhibition mean±SEM	Dose (mg/kg, p.o)	Ulcer Index mean±SEM
Control	0.5% CMC	46.33±0.65	76.17±0.72	0.5% CMC	-	0.5% CMC	0.31±0.12
Diclofenac	10.0	15.17±0.70*	20.50±0.56*	10.0	70.90±0.92	75	5.06±0.20*
5a	15.5	16.17±0.60*	21.83±0.79*,#	15.5	65.42±1.41#	116.6	1.13±0.22*,#
4+1a	10+4.2	16.33±0.80*,#	23.17±1.33*,#	-	-	75+31.3	4.17±0.19*,#
5b	16.9	14.33±0.76*	19.17±0.60*,#	16.9	68.16±1.26	126.7	0.94±0.23#
4+1b	10+5.5	15.67±1.05*,#	20.33±0.61*,#	-	-	75+41.6	4.08±0.34*,#
5c	16.4	20.50±0.67*,#	27.50±2.26*	16.4	59.95±1.18#	123.2	1.31±0.24*,#
4+1c	10+5.1	16.83±0.87*,#	22.50±0.88*,#	-	-	75+38.0	4.33±0.12*
5d	16.5	15.50±0.76*	21.17±0.48*,#	16.5	65.17±0.99#	123.7	1.06±0.35*,#
4+1d	10+5.1	17.17±0.60*,#	23.67±1.31*	-	-	75+38.5	3.92±0.39*,#
5e	16.0	13.17±1.17*	16.83±0.79*,#	16.0	71.39±1.90	120.1	0.75±0.36#
4+1e	10+4.7	14.67±0.42*,#	19.17±0.87*,#	-	-	75+35.0	4.00±0.14*,#
5f	16.8	16.67±0.88*	25.50±0.76*,#	16.8	60.95±1.61#	126.2	1.19±0.25*,#
4+1f	10+5.5	15.67±0.56*,#	21.17±0.48*,#	-	-	75+41.1	4.25±0.13*,#
5g	16.6	23.50±0.56*,#	34.17±1.01*,#	16.6	51.00±1.05#	124.7	1.44±0.26*,#
4+1g	10+5.3	17.83±0.75*	24.33±0.92*,#	-	-	75+39.5	4.42±0.44*

*P<0.05 as compared to control, #P<0.05 as compared to Diclofenac (10 mg/kg, p.o.).

in dichloromethane (25 mL) was cooled in an ice salt mixture to -10°C. To this reaction mixture, chloroacetyl chloride (**2**; 0.01 mole) in $CHCl_3$ (25 mL) was added drop wise with constant stirring over a period of 1 h, maintaining the temperature constant. The reaction mixture was stirred further for 5 h, washed with HCl (5%, 3×50 mL), sodium hydroxide (5%, 3×50 mL) and finally with brine solution (2×25mL). The organic layer was dried over anhydrous sodium sulphate, filtered and the solvent was removed under reduced pressure to obtain the corresponding antioxidant chloroacetyl derivative. This general procedure was used starting with different antioxidants **1a-g** to prepare corresponding chloroacetyl derivatives **3a-g**. These derivatives were recrstyallized from petroleum ether and ethyl acetate **(Table I)**.

General procedure for synthesis of Diclofenac-antioxidant mutual prodrugs (5a-g). A mixture of appropriate antioxidant chloroacetyl derivatives (0.01 mole), diclofenac (**4**; 2.96 g, 0.01 mole), TEA (0.01 mole), sodium iodide (0.01 mole) in DMF (25 mL) was stirred overnight at room temperature The reaction mixture was poured into finely crushed ice with stirring and extracted with chloroform (4×25 mL). The combined organic layer was washed with sodium thiosulphate (2%, 3×50 mL), HCl (5%, 3×50 mL), sodium hydroxide (5%, 3×50 mL) and finally with brine solution (2×25 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and the solvent was removed under reduced pressure to obtain semisolid residue, which was chromatographed on silica gel column using petroleum ether:ethyl acetate mixture as eluent. This general procedure was used starting with different antioxidants chloroacetyl derivatives **3a-g** to prepare various diclofenac-antioxidant mutual prodrugs **5a-g**. The final products were obtained as solids and recrystallized from petroleum ether and ethyl acetate **(Table I)**.

Pharmacology

Wistar rats (150-200 g) of both sexes and laca mice (male, 25-35 g) procured from Central Animal House, Panjab University, Chandigarh, India were used. Animals were housed under standard laboratory conditions, allowed free access to food and water until used and fasted 24 h prior to studies.

Unless otherwise stated, the following conditions were employed in all experiments. The test compounds were suspended in 0.5% carboxymethyl-

cellulose (CMC) and administered per orally (p.o.). Control animals were given the corresponding amount of vehicle (0.5%, CMC). The test drugs were administered on molar equivalent basis of diclofenac.

Statistical Analysis

Results were expressed as mean ± SEM. Significance of the difference of the responses to treatment group in comparison to control group was determined by one-way analysis of variance (ANOVA) followed by Dunnet's t-test. $p<0.05$ was considered significant.

Antiinflammatory activity

Antiinflammatory activity was determined by using carrageenan induced rat paw edema model. Rats were divided into different groups and the diclofenac-antioxidant mutual prodrugs were administered to each group. Acute edema was induced in left hind paw of rats by injecting freshly prepared solution of carrageenan (Type IV, 0.1 mL, 1%) under plantar region of left hind paw. In the right paw, saline (1 mL, 0.9%) was injected, which served as control for comparison. The increase in paw volume was measured by using plethysmometer (water displacement, UGO BASILE, Italy) at 2 and 4 h after carrageenan challenge. Percentage change in paw volume was calculated and expressed as the amount of inflammation **(Table II)**[29].

Analgesic activity

Analgesic activity was determined by using abdominal writhing assay **(Table II)**. Mice were divided into different groups containing 6 animals in each group. Writhing response was elicited by intraperitoneal (i.p.) injection of freshly prepared acetic acid solution (1%, 10 mL/kg, i.p.). The number of writhes due to acetic acid was expressed as antinociceptive response. The number of writhes per animal was counted during a 20 min period. Writhings were counted 3 min after the injection of acetic acid solution[30].

$$\% \text{ Inhibition} = (1-Nt/Nc)\times 100$$

where, Nc – number of writhes in control group and

Nt – number of writhes in drug treated group

Antiulcer activity

The fasted animals (rats) were divided into different groups containing 6 animals in each group. Animals were treated with diclofenac (75 mg/kg, p.o.), equimolar doses of diclofenac-antioxidant mutual prodrugs and their physical mixture. Animals were sacrificed 12 h after the treatment. The stomach was removed, opened along greater curvature, washed with saline and observed for the ulcers[31]. The ulcers were scored **(Table II)** as

0 - Normal colored stomach
0.5 - Red coloration
1.0 - Spot ulcers
1.5 - Hemorrhagic streaks
2.0 - Ulcers > 3 but < 5
3.0 - Ulcers > 5

Conclusion

In our attempt to combine antiinflammatory and antioxidant activities, it has been possible to synthesize diclofenac-antioxidant mutual prodrugs as safer NSAIDs using different naturally occurring phytophenols as antioxidant promoieties. Further, these agents were found to possess encouraging results with retention of antiinflammatory and analgesic activity with significant reduction in ulcerogenic side-effects of the parent NSAID. The diclofenac-guaiacol **5a**, diclofenac-eugenol **5b**, diclofenac-vanillin **5d**, diclofenac-sesamol **5e**, conjugates showed maximum antiulcer activity. The absence of gastric damage in all these cases may be attributed to the combined effect of antioxidant activity of the compounds as well as improved physicochemical properties of the prodrugs. Furthermore, diclofenac with antioxidants physical mixture did not effectively reduce the risk of GI side-effects in comparison to their corresponding conjugates. These results suggest that there is a potential advantage in giving such drugs having complementary pharmacological activities, in the form of single chemical entity i.e. mutual prodrugs which are designed with improved physicochemical properties.

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