Research Article

Nimesulide Based Novel Glycolamide Esters: Their Design, Synthesis, and Pharmacological Evaluation

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The nimesulide based novel glycolamide esters were designed and synthesized for the first time *via* a three-step method starting from nimesulide. Structures of the synthesized compounds were confirmed by spectroscopic analysis. All the synthesized compounds were examined for their cytotoxic effects *in vitro*, some of which showed significant cytotoxic activities against HCT-15 human colon cancer cell line.

1. Introduction

Cancer is the second leading cause of death [1] worldwide after cardiovascular diseases, according to WHO. Indeed, lung, breast, stomach, liver, and colorectal cancers cause the most cancer deaths worldwide each year, and therefore discovery and development of suitable agents to treat various types of cancer are highly desirable.

Over the years, glycolamide esters have attracted particular attention as prodrugs of many pharmaceutically important molecules [2–4] because of their ability to undergo quick cleavage in human plasma to deliver the parent drugs. These include glycolamide esters of aspirin [5], ibuprofen [6], niflumic acid [7], and scutellarin [8] that are reported as biolabile prodrugs of these drugs. However, studies have suggested that the rate of plasma catalyzed hydrolysis of glycolamide esters can be altered with the change of substituents on amide nitrogen atom [9–12]. For example, monosubstituted ($-\text{CO}_2\text{CH}_2\text{CONHR}$) or unsubstituted ($-\text{CO}_2\text{CH}_2\text{CONH}_2$) glycolamide esters were found to be more resistant than *N,N*-disubstituted glycolamide esters ($-\text{CO}_2\text{CH}_2\text{CONRR}'$). Indeed, some of these stable prodrugs were found to behave like new chemical entities (NCEs) and have shown promising pharmacological effects comparable to the parent drugs. For

example, Colfenamate (Figure 1), a glycolamide ester, has shown good analgesic and anti-inflammatory properties [13]. These observations and our interest in novel cytotoxic agents prompted us to synthesize and evaluate a library of new glycolamide esters **A** (Figure 1).

The design of our target molecules **A** was based on the structural modifications of nimesulide [14–20]. We anticipated that combining the structural features of nimesulide and glycolamide esters in a single molecule may afford novel template for the design and synthesis of new anticancer agents. Notably, the cyclooxygenase-2 (COX-2) inhibitor nimesulide is known to possess anticancer properties [21, 22] whereas the glycolamide esters are known to impart favorable pharmacological properties. Herein we report the synthesis and *in vitro* cytotoxic effects of a series of novel glycolamide esters **A** derived from nimesulide. To the best of our knowledge, synthesis and pharmacological evaluation of glycolamide esters derived from nimesulide are not known in the literature.

2. Experimental

2.1. General Methods. Melting points were determined by open glass capillary method on a Cintex melting point

FIGURE 1: Known glycolamide ester Colfenamate, COX-2 inhibitor nimesulide, and new glycolamide esters A.

apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer spectrometer using KBr pellets. ^1H NMR spectra were recorded on a Bruker ACF-300 machine and a Varian 300 and 400 MHz spectrometer using CDCl_3 or $\text{DMSO}-d_6$, with reference to tetramethylsilane as an internal reference. ^{13}C NMR spectra were recorded on a 75 MHz spectrometer. Elemental analyses were performed by Varian 3 LV analyzer series CHN analyzer. Mass spectra were recorded on a Jeol JMC D-300 instrument by using electron ionization at 70 eV. All reactions were monitored by TLC on precoated silica gel plates. Column chromatography was performed on 100–200 mesh silica gel (SRL, India) using 10–20 times (by weight) of the crude product. All the carboxylic acids used are commercially available.

2.2. Synthesis of 2-Chloro-N-(4-methanesulfonylamino-3-phenoxy-phenyl)-acetamide (2). 3.6 mmol (1 g) of compound (1) was taken into a round bottomed flask. To this 20 mL of CHCl_3 and 0.6 mL of Et_3N were added. This mixture was stirred and then cooled to 0°C using an ice bath. To this mixture 3.6 mmol (0.5 mL) of α -chloroacetyl chloride was added drop wise. The mixture was then cooled to room temperature and stirring was continued for an additional 30 minutes. After completion of the reaction as indicated by TLC, the mixture was diluted with 10–15 mL of cold water, which was then extracted with CHCl_3 (3×20 mL). The organic layers were collected, combined, washed with water, dried over anhydrous Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product obtained was recrystallised from aqueous EtOH to give the titled compound as an off-white solid; mp $135\text{--}136^\circ\text{C}$; R_f : 0.45 (CHCl_3 : ethyl acetate = 9 : 1); IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 3325, 3265, 1665, 1611, 1589; ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.30 (s, 1H, NHCO, D_2O exchangeable), 9.25 (s, 1H, NH, D_2O exchangeable), 7.50–7.40 (m, 2 H), 7.38–7.26 (m, 2 H), 7.21–7.14 (m, 2 H), 7.10 (d, J 8.8 Hz, 2H), 4.20 (s, 2 H), 3.00 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3): δ 164.7, 155.4, 148.2, 134.8, 130.2, 124.7, 124.4, 123.1, 118.8, 115.6, 110.1, 52.7, 39.4; MS (m/z): 355 (M^+ , 100%), 357.2 ($\text{M}^+ + 2$, 33%); Elemental analysis: found: C, 50.84; H, 4.02; N, 7.73; $\text{C}_{15}\text{H}_{15}\text{ClN}_2\text{O}_4\text{S}$ requires C, 50.78; H, 4.26; N, 7.90.

2.3. General Procedure for the Synthesis of Glycolamide Esters (4a-l). A mixture of *N*-chloroacetamide (0.01 mol), an appropriate acid (0.01 mol), potassium iodide (0.001 mol), triethylamine (0.011 mol), and *N,N*-dimethylformamide (10 mL) was stirred for 15–45 min (depending on the acid derivative) at 90°C . The reaction mixture was poured into

water (50 mL) and extracted with ethyl acetate (3×50 mL). The combined organic extracts were washed with 2% aqueous sodium bicarbonate (50 mL) and water (3×50 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 , and evaporated under reduced pressure. The crude product obtained was purified by column chromatography to get the corresponding glycolamide esters (4a-l).

2.4. MTT Assay for Cytotoxicity. The viability of the cells was assessed by MTT [3, 4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay, which is based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product. Etoposide, a known anticancer drug, was used as a reference compound in this assay. Cells (1×10^4) were plated in a 96-well plate. After 24 h, they were treated with different concentrations (0–25 μM) of different test compounds diluted appropriately with culture media for 48 h. Cells grown in media containing equivalent amount of DMSO served as positive control and cells in medium without any supplementation were used as negative control. After the treatment, media containing compound were carefully removed by aspiration. 100 μL of 0.4 mg/mL MTT in PBS was added to each well and incubated in the dark for 4 h. 100 μL of DMSO was added to each well and kept in an incubator for 4 h for dissolution of the formed formazan crystals. The amount of formazan was determined by measuring the absorbance at 540 nm using an ELISA plate reader. The data were presented as percent dead cells, whereas absorbance from nontreated control cells was defined as 100% live cells. The percent of dead cells was plotted (y -axis) against concentration (x -axis) of compounds, where IC_{50} values could be interpolated from the graph.

3. Results and Discussion

The key starting material, that is, 2-chloro-*N*-(4-methanesulfonylamino-3-phenoxy-phenyl)-acetamide (2) required for our synthesis was prepared *via* chloroacetylation of *N*-(4-amino-2-phenoxyphenyl) methane sulfonamide [14] (1, obtained from nimesulide) in dry CHCl_3 in presence of Et_3N (Scheme 1).

The chlorocompound 2 was well characterized by using MS, IR, ^1H , and ^{13}C NMR spectroscopic techniques. For example, the IR spectrum showed absorption at 1665 ($\text{C}=\text{O}$ stretching), 3325, and 3265 cm^{-1} (NH stretching) whereas ^1H NMR spectrum recorded in $\text{DMSO}-d_6$ displayed two singlets at δ 4.20 and 2.96 ppm due to CH_2 and CH_3 groups, respectively. The presence of NH groups was further

SCHEME 1: Synthesis of key starting material **2**. SCHEME 2: The reaction of chloro compound **2** with carboxylic acids **3**.

indicated by the disappearance of two peaks at δ 10.46 and 9.22 ppm in D_2O exchange experiment. Moreover, the ^{13}C signals at 164.7, 52.7, and 39.4 ppm indicated the presence of amide carbonyl, CH_2 and CH_3 groups, respectively. Having prepared the starting material **2**, we then treated this chloroderivative with commercially available carboxylic acids in the presence of catalytic amount of *N*, *N*-dimethyl-4-aminopyridine [9] using Et_3N as a base (Scheme 2). To our surprise, instead of the desired glycolamide esters (**4**) we isolated a white solid with the melting point 264–265°C irrespective of acid partner employed. Based on the spectral data (IR, 1H & ^{13}C NMR, and MS) the isolated solid was confirmed as 2-(4-dimethylamino-pyridin-1-yl)-*N*-(4-methanesulfonylamino-3-phenoxy-phenylcarbamoyl)-methyl ester (**5**). It was evident that DMAP reacted better than the acid **3** with the chloro compound **2** perhaps due to its predominant role as a nucleophile rather than a base under the reaction conditions employed. We therefore decided to omit its use in our subsequent studies.

We then performed the reaction of **2** with **3** under a modified reaction conditions, that is, in the presence of potassium iodide and Et_3N . To our satisfaction the desired product **4** was isolated in good yields and the results are summarized in Table 1. It is evident from Table 1 that both aliphatic and aromatic acids participated well in the present reaction and a range of nimesulide based glycolamide esters (**4**) were prepared by using this methodology. All the compounds synthesized were well characterized by spectral data. For example, compound **4a** showed IR signals at 1742 (ester carbonyl) and 1677 cm^{-1} (amide carbonyl), respectively. The appearance of two singlets at δ 2.20 for 3H and δ 4.64 for 2H in 1H NMR spectrum of **4a** confirmed the presence of $-COMe$ and CH_2 groups, respectively. In ^{13}C NMR appearance of 15 signals was highly consistent with the 15 nonequivalent carbon atoms of the compound **4a**. Moreover, the ester and amide carbonyls appear at δ 169.9 and 165.5 ppm, whereas the CH_2 group appeared at δ 62.4 ppm.

All the glycolamide esters **4** synthesized were tested *in vitro* against HCT-15 human colon cancer cell line based on an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Table 2) [23]. Defined as a cancer from uncontrolled cell growth in the colon or rectum (parts of the large intestine), or in the appendix, colon cancer (commonly known as colorectal cancer or bowel cancer) is the third most commonly diagnosed cancer in the world. It was estimated in 2008 that worldwide 1.23 million new cases of colorectal cancer were clinically diagnosed, and that it killed 608,000 people [24]. This prompted us to focus on colon cancer. In our present assay, the percentage of cell death was measured for each compound at various concentrations and finally the IC_{50} (half maximal inhibitory concentration) values were determined to measure the cytotoxic activities. The IC_{50} is inversely proportional to the cytotoxicity of a compound; that is, the lower the IC_{50} value is, the higher the activity is. In general, most of the compounds were found to be active against the HCT-15 colon cancer cell line at 25 μM . Notable among them are **4d** (Entry 4, Table 2), **4f** (Entry 6, Table 2), **4g** (Entry 7, Table 2), **4i** (Entry 9, Table 2), **4j** (Entry 10, Table 2), **4k** (Entry 11, Table 2), and **4l** (Entry 12, Table 2) that showed >40% inhibition at 25 μM . It is evident that an aryl group attached to the ester carbonyl moiety was generally more favorable than an alkyl group for activity (e.g., **4a-b** versus **4c-f**). While insertion of an olefin moiety between the ester carbonyl and the aryl group was tolerated (e.g., **4g**), a naphthylmethyl group (e.g., **4h**) attached to the carbonyl moiety decreased the activity. However, a benzyl group having substituent at *p*- or *o*-position (e.g. **4j-l**) was favorable for activity. A graphical presentation of *in vitro* cytotoxic activities of compound **4l** against HCT-15 human colon cancer cell line is shown in Figure 2. The IC_{50} values of most active compounds, that is, **4j**, **4k**, and **4l**, were found to be 24.40, 22.40, and 18.92 μM , respectively. Thus, the compound **4l** (calculated average $LogP = 2.83 \pm 0.46$ and average $logS = -6.04$ versus 1.75 ± 0.26 and -4.24 , resp., of compound **1** [25]) was identified as the best active compound

TABLE 1: Synthesis of nimesulide based glycomide esters **4**.^a

<div style="text-align: center;"> </div>				
Entry	RCOOH (3) R =	Products (4)	Time (min)	% yield ^b
(1)	—CH ₃ 3a	4a	30	91
(2)	—CH ₂ CH ₃ 3b	4b	30	95
(3)	3c	4c	20	80
(4)	3d	4d	30	90
(5)	3e	4e	30	70

TABLE 1: Continued.

(6)	3f	4f	15	75
(7)	3g	4g	10	98
(8)	3h	4h	30	78
(9)	3i	4i	45	80
(10)	3j	4j	15	92
(11)	3k	4k	30	90

TABLE 1: Continued.

(12)	3l	4l	15	90
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^aAll the reactions were performed using chlorocompound **2** (0.01 mmol) and acid **3** (0.01 mol) in the presence of NaI (0.001 mol), Et₃N (0.011 mmol), and DMF (15 mL) at 90°C.

^bIsolated yield.

TABLE 2: *In vitro* cytotoxic activity of compound **4** against HCT-15 human colon cancer cell line.

Entry	Compounds	% of cell death at various concentrations ^a					IC ₅₀ (μM)
		1 μM	2 μM	5 μM	10 μM	25 μM	
1	4a	0.94	6.89	8.15	8.77	20.30	n.d.
2	4b	0.62	7.20	7.50	18.50	28.50	n.d.
3	4c	0.31	12.5	2.50	7.52	31.00	n.d.
4	4d	3.43	4.53	21.69	35.78	49.38	n.d.
5	4e	1.88	6.58	13.16	17.55	28.80	n.d.
6	4f	5.88	14.46	20.22	25.00	42.40	n.d.
7	4g	6.00	7.96	14.46	18.62	41.91	n.d.
8	4h	0.31	7.52	8.77	12.20	19.40	n.d.
9	4i	7.35	12.37	14.95	16.29	48.89	n.d.
10	4j	6.74	9.43	25.61	35.90	51.22	24.40 ± 0.14
11	4k	0	5.96	12.20	30.70	55.79	22.40 ± 1.31
12	4l	21.69	21.93	24.50	30.02	66.05	18.92 ± 0.09

^aAll the values are the average of the experiments done in triplicates. Etoposide (IC₅₀ = 9.85 μM) was used as a reference compound. n.d.: not done.

in this series. Notably, the IC₅₀ value of nimesulide was found to be >150 μM [26] whereas its reduced product did not show significant activities.

Since the colon cancer is common in developed countries, the present class of glycolamide esters has medicinal value. It is worthy to mention that nimesulide is a banned drug in many countries because of its potential liver toxicity. The observed liver toxicity of nimesulide has often been linked to its uncoupling effects on mitochondria and study has shown that nimesulide exerts this effect *via* a protonophoretic mechanism as well as oxidation of mitochondrial NADH and NADPH [27]. The nitro group of nimesulide was thought to be responsible for its protonophoretic and NAD(P)H oxidizing properties as chemical reduction of –NO₂ to –NH₂ completely suppressed these activities. Thus, the present class of compounds that does not contain a nitro group on the –NHSO₂Me bearing phenyl ring is expected to be free from the liver toxicities of nimesulide. We also performed the stability studies using the compound **4l** in the presence of 50 mM tris buffer (pH 7.4) initially where 60% of **4l** was found to remain unchanged after 2 h incubation in buffer. This study indicated that the present class of compounds perhaps is not susceptible towards rapid ester hydrolysis. Moreover, not being the direct prodrug of nimesulide, these compounds

would not provide nimesulide as such after ester hydrolysis. Thus these compounds are expected to be free from the gastric ulceration problem of nimesulide or other NSAIDs (nonsteroidal anti-inflammatory drugs).

Recently, a nimesulide analogue obtained *via* its chemical modification, that is, replacing the phenyl ether moiety by an aryl ether and the nitro group by a cyclohexyl carboxamide moiety, showed suppression of three breast cancer cell proliferation types in a dose dependent manner [21]. While the anticancer molecular target of this compound was not clearly understood, the cytochrome *c* release assay indicated that the apoptosis induced by this compound was mediated through the mitochondria. Due to the structural similarities with this compound, present series of glycolamide esters might follow similar cytochrome *c* dependent mechanisms. Nevertheless, our study indicates that the glycolamide esters derived from nimesulide [28–30] are of further interest.

4. Conclusions

In conclusion, we have successfully accomplished the synthesis of several new nimesulide based glycolamide esters *via* a three-step method starting from nimesulide in good yields. Structures of the synthesized compounds were confirmed

FIGURE 2: Graphical presentation of *in vitro* cytotoxic activity of compound 4I against HCT-15 human colon cancer cell line.

by spectroscopic analysis. All the synthesized compounds were examined for their cytotoxic effects *in vitro*. Some of the compounds showed significant cytotoxic activities against HCT-15 human colon cancer cell line. Overall, the present nimesulide based glycolamide ester framework appeared to be a useful template for the design and identification of novel and potential anticancer agents.

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