Synthesis of furoxan derivatives of diclofenac as potent anti-inflammatory agents with reduced GI toxicity

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A new series of NO donating furoxan derivatives of diclofenac have been synthesized by linking diclofenac to selected furoxan moieties and have been investigated for their anti-inflammatory, analgesic, ulcerogenic, lipid peroxidation, hepatotoxicity, histopathological and NO releasing properties. All the hybrid derivatives are endowed with anti-inflammatory activity comparable to diclofenac but unlike this drug they show reduced GI toxicity. The compounds $4-\{(2-(2-(2,6\text{-dichlorophenylamino})phenyl)acetamido)ethoxy)carbonyl\}-3$-methyl furoxan $12$ having amide-ester linkage and $4-\{(2-(2,6\text{-dichlorophenylamino})phenyl)acetoxy)methyl\}-3$-methyl-furoxan $13a$ having ester linkage show greater anti-inflammatory activity comparable to standard drug diclofenac. These compounds $12$ and $13a$ also show higher gastrointestinal protection in comparison to standard drug diclofenac.

Keywords: Diclofenac, nitric oxide donors, furoxan, anti-inflammatory, ulcerogenicity, lipid peroxidation, hepatotoxic effect, histopathological studies

Nonsteroidal anti-inflammatory drugs (NSAIDs) viz. ibuprofen, naproxen and diclofenac are commonly employed drugs for the treatment of various chronic anti-inflammatory diseases. Among these, diclofenac has been approved in 120 countries since its introduction 36 years ago and ranked among the top 200 drugs with respect to new prescription. NSAIDs therapy effectively reduces the symptoms of many painful arthritic syndromes, but invites adverse gastrointestinal (GI) complications ranging from stomach irritation to life threatening GI ulceration, bleeding, and perforation to more serious small-bowel ulceration$^{1-3}$. It occurs due to the inhibition of cyclooxygenase (COXs) as well as acidic character of NSAIDs themselves$^4$. In order to reduce GI toxicity various COX-2 selective inhibitors viz. celecoxib, rofecoxib have been developed which showed marked anti-inflammatory activity but induce less GI side effect in comparison to non selective COX inhibitors such as aspirin, ibuprofen and diclofenac$^{5,6}$. But selective COX-2 inhibitors have been withdrawn from the market due to their adverse cardiovascular side effects$^7$. Therefore, development of NSAIDs with improved safety profile is still a necessity.

One interesting innovative approach relies on the incorporation of a nitric-oxide (NO) releasing moiety into the structure of established NSAIDs molecules. Such NO-donating NSAIDs are emerging as a new class of effective anti-inflammatory compounds having an enhanced safety profile$^{8,9}$. The rationale behind this drug design is based on the biological proprieties of NO. It has been reported that an increased generation of endothelial NO or release of NO from a nitric oxide donor drug produces beneficial effects such as reduction of blood pressure and prevention of atherosclerosis$^{10}$. At nanomolar concentrations, NO reversibly activates soluble guanylate cyclase (sGC) by 400 folds, catalyzing the conversion of guanosine triphosphate to cyclic guanosine monophosphate (cGMP)$^{11}$. Elevation of cGMP relaxes smooth muscles in blood vessels, inhibits platelet aggregation and blocks the adhesion of white cells to blood vessels walls$^{12}$. Besides these cardiovascular effects, NO is also recognized as a critical mediator of gastrointestinal mucosal defense. NO is able to protect gastric mucosa by a number of mechanisms including promotion of mucus secretion and increased mucosal blood flow resulting in enhanced mucosal resistance to ulceration. NO also increases the ability of mucous cells to undertake healing and repair of the existing ulcers$^{13}$. Encouraged by these observations and in continuation of our ongoing research program$^{14-17}$ to discover new and useful agents for treatment of anti-inflammatory
disease, we report herein the synthesis and pharmacological profile of furoxan derivatives of diclofenac as potential NO donor drugs.

**Result and Discussion**

The reaction sequence leading to the formation of the desired heterocyclic compounds are outlined in Scheme I and Scheme II. Compounds 1 (Ref 18), 2 (Ref 19), 3 (Ref 20), 4 (Ref 21), 5a-b (Ref 22) and 6a-b (Ref 22) were synthesized according to methods reported in literature. Intermediate compounds 8 and 11 were synthesised according to literature method by slightly modifying reaction conditions.23

Substituted furoxans were synthesized from crotonaldehyde and cinnamaldehyde (Figure 1). Compound 9 was synthesized by treating 2-[2-(2,6-dichlorophenylamino)phenyl]acetyl chloride 7 with ethylene glycol to form an intermediate 2-hydroxyethyl-2-[2-(2,6-dichlorophenylamino)phenyl]acetate which on further treatment with 4-(chlorocarbonyl)-3-methyl furoxan 6a resulted in the formation of 4-[(2-(2-(2,6-dichlorophenylamino)phenyl)acetoxy)methyl]-3-methyl-furoxan 9. The ethyl ester of diclofenac 10 on treatment with ethanol amine forms an intermediate 2-[2-(2,6-dichlorophenylamino)phenyl]-N-(2-hydroxyethyl)acetamide 11 which on further treatment with intermediate 6a resulted in the formation of [(2-(2-(2,6-dichlorophenylamino)phenyl)acetamido)ethoxy]carbonyl]-3-methyl-furoxan 12. The final compounds 4-[(2-(2,6-dichlorophenylamino)phenyl)acetoxy]methyl]-3-substituted-furoxan 13a-b of the series were prepared by treating intermediate 7 with 4-(hydroxymethyl)-3-methyl/phenyl furoxan in presence of triethyl amine (Scheme I). The compounds 4-[(2-(2-(2,6-dichlorophenylamino)phenyl)acetyl)hydrazono]methyl]-3-substituted-furoxan 14a-b were prepared by treating 2-[2-(2,6-dichlorophenylamino)phenyl]acetohydrazide with 4-formyl-3-methyl/phenyl-furoxan in presence of concentrated sulphuric acid. The compound 4-[(2-(2,6-dichlorophenylamino)phenyl)acetohydrazide and 4-(chlorocarbonyl)-3-substituted-furoxan 6a-b in presence of triethylamine. The compound 4-[5-(2-(2,6-dichlorophenylamino)benzyl]-1,3,4-oxadiazol-2-yl]-3-methyl-furoxan 16 was synthesized by refluxing 2-[2-
AMIR et al.: FUROXAN DERIVATIVES OF DICLOFENAC

991

The purity of the compounds was checked by elemental analyses and spectral data. Both the analytical and spectral data (IR, $^1$H and $^{13}$C NMR, and mass spectroscopy) of the compounds were in full harmony with the proposed structures. The results of elemental analysis (C, H and N) were within ±0.3% of the theoretical values.

Biological Studies

The synthesized compounds were evaluated for their anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation, hepatotoxic and histopathological properties. The Wistar rats and albino mice used in the present study were housed and kept with the Hamdard University Animal Care Unit, which applies the guidelines in accordance with the rules laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. All the test compounds and standard drug were administered in the form of solution (0.5% w/v carboxymethyl cellulose as a vehicle) by an oral route. Each group consisted of six animals. All the animals were procured from the CPCSEA and maintained in colony cages at 25 ± 2°C, with relative humidity of 45–55%, under a 12 h light and dark cycle and were fed a standard animal feed. All the animals were acclimatized for a week before use. The anti-inflammatory activity of the test compounds were compared with the control. The analgesic, ulcerogenic and lipid peroxidation activities were compared with the standard drug diclofenac. Data were analysed by student’s $t$ test for $n = 6$.

Measurement of nitric oxide release

All the compounds 9, 12, 13a-b, 14a-b, 15a-b and 16 were tested for their NO releasing properties in vitro in both phosphate buffer of pH 7.4 and 0.1N HCl of pH 1 by using Griess reagent. The reaction was carried out in the presence of L-cysteine as a source of the SH group. It was found that a reduced thiol group from endogenous L-cysteine could mediate the release of NO from furoxan derivatives. The amount of NO release from test compounds was measured relative to NO released from standard sodium nitrite solution and calculated as amount of
NO released (mol/mol) and listed in Table I. Linker groups which attach furoxan with diclofenac had significant effect on NO releasing capacity of compounds. The compound 12 having an amide-ester linkage and a methyl group in the furoxan ring and compound 13a having an ester linkage and a methyl group in the furoxan ring showed maximum nitric oxide releasing capacity. The compound 14b having amide-Schiff base linkage showed minimum nitric oxide releasing capacity. Other compound of the series having an amide-amide linkage 15a-b and 1,3,4-oxadiazole spacers 16 showed moderate nitric oxide releasing capacity. On the other hand the tested compounds were not able to release NO at pH 1, which suggests that NO donating furoxan derivatives of diclofenac are weakly hydrolysed in the gastric lumen and this confirmed that the suggested gastro protective action of NO is mediated systemically (Table I).

**Anti-inflammatory activity**

All the synthesized compounds were screened for their anti-inflammatory activity by carrageenan induced rat paw edema method as described by Winter et al. The results revealed that compounds 12, 13a and 15a exhibited excellent anti-inflammatory activity (85.97%, 80.44% and 79.16% respectively), at an equimolar oral dose relative to 10 mg/Kg diclofenac which showed 77.41% inhibition after 4 h. Compounds 13b and 15b showed anti-inflammatory activity comparable to standard drug diclofenac i.e. 75.83% and 73.06% respectively. Compound 14a also showed good anti-inflammatory activity whereas compounds 9, 14b and 16 showed lesser degree of activity. Thus it was concluded that furoxan derivatives having amide-ester linkage or ester-ester linkage or ester linkage showed very good activity. These results indicate that the incorporation of NO donating furoxan moieties to diclofenac have not only retained but showed enhanced anti-inflammatory activity (Table II).

**Analgesic activity**

The compounds which showed significant anti-inflammatory activity (more than 65%) were further tested for their analgesic effect at the same oral dose as used for the anti-inflammatory activity. The analgesic activity was carried out by tail immersion method and the results are reported as % analgesia (Table III). Analgesic activity of furoxan derivatives showed 57.15% to 79.05% whereas standard drug diclofenac showed 74.49% activity. Compound 13a having ester linkage with a methyl group at furoxan ring showed maximum activity (79.05%). Other compounds 12, 13b and 15a also showed very good activity ranging from 65.40% to 74.19%. Compound 14a having amide-Schiff base linkage with a methyl group at furoxan ring showed poor activity (57.15%).

**Ulcerogenic activity**

The compounds 9, 12, 13a-b and 15a which showed significant anti-inflammatory and good analgesic activity were further tested for their acute ulcerogenicity by the method of Cioli et al. (Table III). The compounds were tested at an equimolar oral dose relative to 30 mg/Kg diclofenac. The tested compounds showed severity index ranging from 0.250 ± 0.11 to 0.583 ± 0.08 whereas the standard drug diclofenac showed severity index of 0.666 ± 0.10. Compound 12 having amide-ester linkage and a methyl group at furoxan ring showed minimum ulcerogenicity (severity index 0.250 ± 0.11),

<table>
<thead>
<tr>
<th>Compd</th>
<th>Amount of NO release (mol/mol)</th>
<th>Compd</th>
<th>Amount of NO release (mol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0.54 ± 0.033</td>
<td>14b</td>
<td>0.21 ± 0.039</td>
</tr>
<tr>
<td>12</td>
<td>0.65 ± 0.082</td>
<td>15a</td>
<td>0.43 ± 0.043</td>
</tr>
<tr>
<td>13a</td>
<td>0.62 ± 0.061</td>
<td>15b</td>
<td>0.31 ± 0.061</td>
</tr>
<tr>
<td>13b</td>
<td>0.53 ± 0.026</td>
<td>16</td>
<td>0.35 ± 0.047</td>
</tr>
<tr>
<td>14a</td>
<td>0.40 ± 0.038</td>
<td></td>
<td>0.31 ± 0.061</td>
</tr>
</tbody>
</table>

Table II — Anti-inflammatory activity of furoxan derivatives 9, 12, 13a-b, 14a-b, 15a-b and 16

<table>
<thead>
<tr>
<th>Compd</th>
<th>Anti-inflammatory activity % inhibition</th>
<th>Compd</th>
<th>Anti-inflammatory activity % inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 3 h</td>
<td></td>
<td>After 3 h</td>
</tr>
<tr>
<td>9</td>
<td>54.71 ± 0.87</td>
<td>14b</td>
<td>54.89 ± 1.07</td>
</tr>
<tr>
<td>12</td>
<td>72.28 ± 0.73</td>
<td>15a</td>
<td>75.46 ± 1.04</td>
</tr>
<tr>
<td>13a</td>
<td>78.07 ± 0.71</td>
<td>15b</td>
<td>70.83 ± 0.65</td>
</tr>
<tr>
<td>13b</td>
<td>71.01 ± 0.54</td>
<td>16</td>
<td>59.78 ± 0.74</td>
</tr>
<tr>
<td>14a</td>
<td>64.49 ± 0.77</td>
<td></td>
<td>73.80 ± 0.72</td>
</tr>
</tbody>
</table>

Test compounds and diclofenac were tested at 10 mg/kg body weight

*aMean ± SEM, n = 6.

Significance levels compared to the control: *p < 0.5, *p < 0.05, *p < 0.001, *p < 0.0001
whereas compounds 13a and 15a having an ester and an amide-amide linkage also showed lesser ulcerogenicity (severity index 0.333 ± 0.10 and 0.416 ± 0.15 respectively). Thus, the results indicated that nitric oxide released from the diclofenac-furoxan derivatives have a significant effect on ulcerogenic activity (Figure 2). The decreased severity index may be due to the release of NO that increased mucosal blood flow resulting in enhanced mucosal resistance to ulceration and/or an enhanced ability of the NO donating furoxan derivatives to cross the gastric mucosal layer prior to the subsequent release of NO.

Lipid peroxidation activity

All the compounds screened for ulcerogenic activity were also analyzed for lipid peroxidation according to the method of Ohkawa et al.29 The lipid peroxidation was measured as nanomoles of malondialdehyde (MDA/100mg) of gastric mucosa tissue. Diclofenac exhibited high lipid peroxidation 7.15 ± 0.06 whereas the control group showed 3.46 ± 0.10. It was found that all the furoxan derivatives showing lower ulcerogenic activity also showed reduction in lipid peroxidation. The compound 12 having amide-ester linkage and compound 13a having ester linkage showed maximum reduction 3.58 ± 0.10 and 4.70 ± 0.07 respectively whereas compound 9 having ester-ester linkage showed minimum reduction 6.67 ± 0.09 nmol MDA/100 mg tissue. Thus these studies showed that the synthesized compounds have inhibited the induction of gastric mucosal lesions and the results further suggested that their protective effect might be related to the inhibition of lipid peroxidation in the gastric mucosa due to release of nitric oxide from furoxan derivatives (Table III).

Hepatotoxic and histopathological activity

Furoxan derivatives 12 and 13a showing potent anti-inflammatory and analgesic activities with reduced ulcerogenicity and lipid peroxidation were further studied for their hepatotoxic effect. Assessment of liver function such as serum glutamate oxaloacetate transaminase (SGOT) and serum

Table III — Analgesic, ulcerogenic and lipid peroxidation activities of synthesized furoxan derivatives 9, 12, 13a, 13b, 14a and 15a

<table>
<thead>
<tr>
<th>Compd</th>
<th>Pre-treatment/normal 0 h</th>
<th>Post-treatment/after 4 h</th>
<th>% Inhibition</th>
<th>Ulcerogenic activity (Severity index)</th>
<th>nmol MDA content/100 mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>1.55 ± 0.01</td>
<td>2.56 ± 0.02</td>
<td>65.40 ± 0.97</td>
<td>0.583 ± 0.08</td>
<td>6.67 ± 0.09b</td>
</tr>
<tr>
<td>12</td>
<td>1.72 ± 0.01</td>
<td>2.98 ± 0.01</td>
<td>70.71 ± 0.98</td>
<td>0.250 ± 0.11b</td>
<td>3.58 ± 0.10b</td>
</tr>
<tr>
<td>13a</td>
<td>1.69 ± 0.01</td>
<td>2.86 ± 0.02</td>
<td>79.05 ± 1.22</td>
<td>0.333 ± 0.10c</td>
<td>4.70 ± 0.07d</td>
</tr>
<tr>
<td>13b</td>
<td>1.47 ± 0.01</td>
<td>2.52 ± 0.02</td>
<td>74.19 ± 1.79</td>
<td>0.500 ± 0.00a</td>
<td>5.97 ± 0.08d</td>
</tr>
<tr>
<td>14a</td>
<td>1.55 ± 0.01</td>
<td>2.44 ± 0.01</td>
<td>57.15 ± 0.97</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15a</td>
<td>1.75 ± 0.01</td>
<td>3.09 ± 0.01</td>
<td>70.15 ± 1.50a</td>
<td>0.416 ± 0.15</td>
<td>5.72 ± 0.11a</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.000 ± 0.00</td>
<td>3.46 ± 0.10</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1.43 ± 0.00</td>
<td>2.50 ± 0.01</td>
<td>74.49 ± 0.96</td>
<td>0.666 ± 0.10</td>
<td>7.15 ± 0.06</td>
</tr>
</tbody>
</table>

Test compounds and diclofenac sodium were tested at 10mg kg⁻¹ body weight.

Mean ± SEM, n = 6.

Significance levels compared to the standard drug: a: p < 0.5, b: p < 0.05, c: p < 0.001, d: p < 0.0001

Figure 2 — Gastric ulcer which is induced by diclofenac drug was reduced by linking NO releasing furoxan moieties with diclofenac
glutamate pyruvate transaminase (SGPT) were estimated by a reported method\(^3\). The alkaline phosphatase, total protein and total albumin were measured according to reported procedures\(^\text{31,32}\) as shown in Table IV. Activities of liver enzyme SGOT, SGPT, alkaline phosphatase, total protein and total albumin were less than for the standard drug diclofenac. Histopathological studies were also carried out by reported methods\(^\text{33}\). The histopathological studies of the liver samples do not show any significant pathological changes in comparison to standard drug diclofenac (Figure 3). No hepatocyte necrosis or degeneration was seen in any of the samples.

**Experimental Section**

The melting points were determined in open capillary tubes in a Hicon melting point apparatus and are uncorrected. Elemental analysis (C,H,N,S) was performed on the CHNS Elimentar (Analysen...
Systine, GmbH) Germany Vario EL III instrument. FTIR spectra were recorded as KBr pellets on a Jasco FT-IR 410 spectrometer and frequency was expressed in cm\(^{-1}\). \(^1\)H NMR spectra were recorded on a Bruker DPX 400 NMR spectrometer. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS); coupling constants (J) are reported in Hertz, and refer to apparent peak multiplicities, and may not necessarily be true coupling constants. Mass spectra were measured on a Jeol SR-102 (FAB) mass spectrometer. Unless otherwise specified, all reactions were carried out in oven-dried glassware, and commercially available starting materials were used without further purification.

**Synthesis of 4-[(2-(2-(2,6-dichlorophenylamino)-phenyl)acetamido)ethoxy]carbonyl]-3-methyl-furoxan, 9**

Ethylene glycol (0.015 mol) and dry CH\(_2\)Cl\(_2\) (20 mL) were slowly mixed together at RT. 2-[2-(2,6-Dichlorophenylamino)phenyl]acetil chloride 7 (10 mmol) in dry CH\(_2\)Cl\(_2\) (5 mL) was added drop-wise to this mixture with continuous stirring under ice cold conditions. The stirring was continued for 5 h and the reaction mixture was poured into ice cold water. The organic layer was separated, washed twice with water, dried and distilled under reduced pressure. The residue thus obtained was purified by recrystallization from petroleum ether. A solution of 4-(chlorocarbonyl)-3-methyl furoxan 6a (10 mmol) in THF (3 mL) was added drop-wise to a stirred mixture of intermediate 11 in dichloromethane (60 mL) in the presence of triethylamine (1 mL). The stirring was continued for 5 h, after which the reaction mixture was poured into water. The organic layer was separated, washed twice with water dried with anhydrous sodium sulphate and distilled under reduced pressure. The residue thus obtained was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compound. Yield 58%. m.p.80-82°C. IR (KBr): 3288 (NH), 1743 (C=O), 1721 (C=O); MS: m/z 355 (M\(^+\)), 353 (M\(^+\)+2). Anal. Calcd for C\(_{20}\)H\(_{17}\)Cl\(_2\)N\(_2\)O\(_6\): C, 51.63; H, 3.90; N, 12.04. Found: C, 51.43; H, 3.74; N, 11.92%.

**General method for synthesis of 4-[(2-(2-(2,6-dichlorophenylamino)phenyl)acetamido)ethoxy]carbonyl]-3-methyl-furoxan, 13a-b**

An equimolar mixture of 4-(hydroxymethyl)-3-methyl furoxan/4-(hydroxymethyl)-3-phenyl furoxan (10 mmol) and triethylamine (10 mmol) was dissolved in dry toluene (20 mL) at 0°C with stirring. To this solution 2-[2-(2,6-dichlorophenylamino)phenyl]acetil chloride 7 was added drop-wise with continuous stirring. The reaction mixture was further stirred for 4-5 h and then poured into ice cold water. The organic layer was separated, washed with water, dried and distilled.
under reduced pressure. The separated solid thus obtained was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford the title compounds.

4-[(2-(2-(2,6-Dichlorophenylamino)phenyl)acetoxy)methyl]-3-methyl-furoxan, 13a: Yield 65%. m.p.91-93°C. IR (KBr): 3233 (NH), 1731 (C=Oamide), 1584 cm⁻¹ (C=N); ¹H NMR: δ 7.07-7.52 (m, 7H, 6ArH and 1NH), 6.39 (d, 1H, ArH), 4.58 (s, 2H, OCH₂); ¹³C NMR: δ 171.24 (C=O), 152.43, 140.25, 135.50, 130.89, 130.44, 129.08, 127.97, 125.13, 124.87, 124.31, 123.12, 109.55, 35.77 (CH₂); MS: m/z 436 (M⁺), 472 (M⁺+2). Anal. Calcd for C₁₈H₁₃Cl₂N₂O₄: C, 51.27; H, 3.52; N, 10.11%. Found: C, 52.76; H, 3.52; N, 10.11%.

4-[(2-(2-(2,6-Dichlorophenylamino)phenyl)acetoxy)methyl]-3-phenyl-furoxan, 13b: Yield 58%. m.p.58-60°C. IR (KBr): 3313 (NH), 1712 (C=Oamide), 1594 cm⁻¹ (C=N); ¹H NMR: δ 7.10-7.80 (m, 12H, 11ArH and 1NH), 6.53 (d, 1H, ArH), 4.16 ( s, 2H, CH₂CONH), 8.16 (s, 1H, N=CH), 6.93-7.45 (m, 7H, 6ArH), 2.53 (s, 3H, CH₃); ¹³C NMR: δ 171.60 (C=O), 146.26, 143.31, 139.32, 135.27, 128.34, 127.83, 127.09, 126.45, 125.72, 121.73, 119.73, 110.11, 36.71 (CH₂), 9.08 (CH₃); MS: m/z 420 (M⁺), 422 (M⁺+2). Anal. Calcd for C₁₈H₁₅Cl₂N₂O₄: C, 51.44; H, 3.60; N, 16.66. Found: C, 51.27; H, 3.42; N, 16.48%.

General method for synthesis of 4-[(2-(2-(2,6-dichlorophenylamino)phenyl)acetyl)-hydrazono)methyl]-3-substituted-furoxan, 15a-b

2-[2-(2,6-Dichlorophenylamino)phenyl] acetohydrazide (10 mmol) was dissolved in dry toluene (20 mL) and triethylamine (10 mmol) was added to it. 4-(Chlorocarbonyl)-3-methyl/phenyl-furoxan in dry toluene (5 mL) was added to the solution drop-wise with continuous stirring. The reaction mixture was further stirred for 4-5 h at RT and then was poured into ice cold water. The organic layer was separated, washed with water, dried and distilled under reduced pressure. The separated solid thus obtained was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford pure compounds.

4-[(2-(2-(2,6-Dichlorophenylamino)phenyl)acetyl)-hydrazino)methyl]-3-methyl-furoxan, 14a: Yield 65%. m.p.102-104°C. IR (KBr): 3239, 3243 (NH), 1704, 1680 (C=O), 1611 cm⁻¹ (C=N); ¹H NMR: δ 8.68 (s, 1H, CONH), 8.16 (s, 1H, CONH), 6.93-7.45 (m, 7H, 6ArH and 1NH), 6.53 (d, 1H, ArH), 4.16 ( s, 2H, CH₂CO), 2.53 (s, 3H, CH₃); ¹³C NMR: δ 174.52 (CCONS), 171.60 (CConH). 146.17, 143.21, 139.22, 135.29, 128.89, 128.37, 127.81, 127.29, 126.54, 121.76, 119.71, 110.17, 36.69 (CH₂), 9.23 (CH₃); MS: m/z 436 (M⁺), 438 (M⁺+2). Anal. Calcd for C₁₈H₁₅Cl₂N₂O₄: C, 49.56; H, 3.47; N, 16.05. Found: C, 49.37; H, 3.29; N, 15.87%.
4-[2-(2-(2,6-Dichlorophenylamino)phenyl)acetyl]-3-substituted-furoxan, 15b: Yield 65%. m.p.110-12°C. IR (KBr): 3248, 3254 (NH), 1684, 1689 (C=O), 7.18-8.80 (m, 13H, 12ArH and 1NH), 4.92 (s, 2H, CH2CO); 13C NMR: δ 174.12 (C=O), 155.55, 149.36, 142.92, 137.86, 135.84, 132.32, 131.43, 130.87, 128.92, 128.25, 127.99, 125.26, 124.37, 122.06, 118.14, 109.25, 38.11 (CH2); MS: m/z 498 (M+), 500 (M+2). Anal. Calcd for C36H26Cl2N2O3: C, 55.44; H, 3.44; N, 14.05. Found: C, 55.31; H, 3.27; N, 13.85%.

Synthesis of 4-[5-(2-(2,6-dichlorophenylamino)benzyl]-1,3,4-oxadiazol-2-yl]-3-methyl-furoxan, 16

2-[2-(2,6-Dichlorophenyl amino)phenyl]acetohydrazide (10 mmol) was dissolved in phosphorus oxychloride (10 mL) and to it was added 4-carboxy-3-methyl furoxan 5a (10 mmol). The reaction mixture was refluxed for 6 h, cooled to RT and poured onto crushed ice. The solution was neutralized with sodium bicarbonate (10%). The solid mass thus separated was washed with water and dried. It was purified by silica gel column chromatography using EtOAc/hexane as eluent to afford the pure compound. Yield 62%. m.p.188-90°C. IR (KBr): 3293 (NH), 1610 (C=N). The compound was then dissolved in DCM and filtered through a nylon filter to remove any trace amount of remaining chlorides. The filtrate was then subjected to gel column chromatography using EtOAc/hexane as eluent to afford the pure compound. Yield 62%. m.p.188-90°C. IR (KBr): 3293 (NH), 1610 (C=N). The compound was then dissolved in DCM and filtered through a nylon filter to remove any trace amount of remaining chlorides. The filtrate was then subjected to gel column chromatography using EtOAc/hexane as eluent to afford the pure compound. Yield 62%.

Conclusions

Nine furoxan derivatives of diclofenac were synthesized and evaluated for their anti-inflammatory and nitric oxide releasing properties. Compounds 9, 12, 13a, 13b and 15a showing high anti-inflammatory and analgesic activity were also tested for their ulcerogenic potential and lipid peroxidation. It was found that compounds 12 and 13a showing high anti-inflammatory activity also exhibited reduced ulcerogenic potential when compared with diclofenac. Furthermore, increased gastro protective activities of these compounds may be due to the release of NO that promoted mucous secretion and increased mucous blood flow resulting in enhanced mucosal resistance to ulceration. Thus the use of the hybrid molecules containing NO releasing furoxan moieties may be a useful approach for the development of GI-safe anti-inflammatory analgesic agents.

Acknowledgment

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