Urea/thiourea derivatives of Gly/Pro conjugated 2,3-dichlorophenyl piperazine as potent anti-inflammatory agents: SAR studies

D M Suyoga Vardhana, H K Kumara, M B Sridhara & D Channe Gowda

Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570 006, India
Department of Chemistry, Ranichannamma University, Vidya Sangama, Belagavi 591 156, India
E-mail: dchannegowda@yahoo.co.in

Received 8 August 2016; accepted (revised) 4 September 2017

Two series of urea/thiourea derivatives of Gly/Pro conjugated 2,3-dichlorophenyl piperazine have been synthesized and characterized as novel anti-inflammatory agents. The results demonstrate that the derivatives 1f, 1g, 1j, 2g, 2j and 2k show good anti-inflammatory properties with IC50 values of 42 ± 0.32 µM, 30 ± 0.51 µM, 65 ± 0.61 µM, 60 ± 0.52 µM, 60 ± 0.22 µM, 57 ± 0.37 µM and 35 ± 0.15 µM respectively. These results reveal that compounds with electron withdrawing moieties (F and Cl) particularly at para position exhibit excellent anti-inflammatory activity compared to the standards indomethacin (IC50 = 58 ± 0.37 µM) and ibuprofen (IC50 = 67 ± 0.43 µM).

Keywords: Amino acids, piperazine, conjugation, urea/thiourea, anti-inflammatory, SAR

Pain and inflammation are among the main problems that significantly influence the lifestyle of millions of people worldwide. There are various therapies available for the treatment of pain and inflammation, but these therapies are not always effective and can cause adverse effects that, in many cases, limit the treatment. Therefore, a major challenge remains for biomedical research: the identification of new compounds for the treatment of pain and inflammation able to induce low or no side effects. Traditional non-steroidal anti-inflammatory drugs, such as aspirin, operate via inhibition of the COX-1 isoenzyme or via inhibition of both COX-1 and COX-2 isoenzymes in drugs such as indomethacin, naproxen, ibuprofen, and flurbiprofen. Inhibition of the COX-2 isoenzyme leads to a reduction in the production of prostanoids, such as prostaglandins and thromboxanes, which are responsible for inflammatory effects. On the other hand, the inhibition of the COX-1 isoenzyme is mainly responsible for gastrointestinal side effects, including ulcerations and bleeding. Therefore, the discovery of new safer anti-inflammatory drugs represents a challenging goal for such a research area.

Indeed, amino acids and their metabolic and physiological ramifications are among the most investigated topics in biomedical science. The synthesis of compounds containing amino acids has attracted the attention of chemists due to their interesting biological activities with low toxicity and ample bioavailability. Furthermore, it is well known that a number of nitrogen containing heterocyclic compounds exhibited a wide variety of biological activities. Among them, piperazine derivatives have owed their importance in many medicinal molecules. Piperazine derivatives were originally used in veterinary medicines which combat parasitic infections in poultry, stimulants at low doses, and cause hallucinations at higher level doses. In addition to the nitrogen functionalities such as urea and thiourea which have been found in broad spectrum of biological activities like antidiabetic, anti-inflammatory, antiviral, antibacterial, antifungal, herbicidal, antituberculosis, and antidepressant activity on central nervous system as well as purification agents for the effluent of organic and inorganic molecules in industrial, agricultural, and mining industries. Recently, some of urea-based compounds act as kinase inhibitors and as novel therapeutics in cancer treatment due to their unique binding mode and kinase inhibition profile. Having more importance in pharmacological applications of urea and thiourea derivatives, there is a possibility to design more effective new drugs for treating various diseases.

List of Abbreviations: Boc: t-Butoxycarbonyl; EDCI: 1-(3-Dimethylaminopropyl)-3-ethyl-carbodiimide.HCl; HOBt: 1-Hydroxybenzotriazole; NMM: N-Methyl morpholine; PBS: Phosphate buffer saline; PZN: 1-(2,3-Dichlorophenyl)piperazine; TFA: Trifluoroacetic acid.
efficient urea and thiourea derivatives as anti-inflammatory agents. Led by the previous facts and coupled with our ongoing project aimed at investigating new bioactive molecules derived from amino acids-heterocyclic conjugates, the present work involves the synthesis of two series of Gly/Pro conjugated 2,3-dichlorophenyl conjugates, having chosen only electron withdrawing groups.

Results and Discussion

The synthetic route for the proposed compounds viz., urea and thiourea derivatives of the conjugates of Gly and Pro is outlined in Scheme I. The physical and spectroscopic data of all the synthesized compounds are in close agreement with our earlier work. In our previous investigations, it was noticed that the presence of electron withdrawing substituents enhances the activity compared to electron donating groups. This formed the basis for the present work wherein, we have chosen only electron withdrawing groups.

All the synthesized compounds were evaluated for their in vitro anti-inflammatory activity on human erythrocyte and the results are presented in Table I. Compounds 1f, 1g, 2j and 2k with an IC₅₀ values of 42 ± 1.11 μg/mL, 30 ± 0.85 μg/mL, 57 ± 1.05 μg/mL and 35 ± 0.92 μg/mL respectively have shown excellent anti-inflammatory activity as compared to standards indomethacin 58 ± 0.58 μg/mL and ibuprofen 67 ± 0.62 μg/mL. Compounds 1j, 1k, 1o and 2g with an IC₅₀ value 65 ± 0.98, 60 ± 0.88, 65 ± 0.68 and 60 ± 1.25 respectively are equipotent with standards. Compounds 1a, 1b, 2a and 2b without any substitution on phenyl ring have shown poor or moderate activity. On this basis, the SAR study revealed that, compounds with electron withdrawing groups (F, Cl and Br) on aromatic phenyl ring of ureas and thioureas show higher activity than the compounds with no substitution. Another interesting feature observed based on the activity profile is that halogen substituents particularly at para position are found to be most active than the compounds with ortho and meta substituents. In general, the SAR study divulged that halogen containing urea and thiourea compounds preferably halogens at para substitution show higher anti-inflammatory activity than the unsubstituted compounds.

Experimental Section

Amino acids used were of L-configuration unless otherwise mentioned. TFA was purchased from Advanced Chem. Tech. (Louisville, KY, USA).

Reagents and Conditions: (a) EDCI, HOBt, NMM, 0°C, overnight at RT; (b) TFA, 45 min; (c) R-C₆H₄-N=C=Z, NMM.

Scheme I — Synthesis of uriedo/thiouriedo derivatives of Gly/Pro conjugated 2,3-dichlorophenyl piperazine
NMM and phenyl isocyanates/isothiocyanates were purchased from Sigma Chemical Co. (St. Louis, MO). Melting points were determined on a Superfit melting point apparatus (India) and are uncorrected. TLC was performed on pre-coated silica gel plates (Kieselgel 60 F254, E. Merck, Germany) with the solvent system comprising chloroform/methanol/acetic acid in the ratio 98:2:3 (Rf a) and 95:5:3 (Rf b) and the compounds on TLC were detected by iodine vapors. Solvent and other chemicals used were of analytical grade. IR spectra of the compounds were recorded on Jasco spectrometer (USA). 1H NMR spectra were obtained on VARIAN 400 MHz instrument (USA) using DMSO-d6 as solvent and the chemical shifts are reported as parts per million (δ, ppm) using TMS as an internal standard. Mass spectra were obtained on Bruker (model HP-1100) (USA) electrospray mass spectrometer.

**Conjugation of Gly/Pro to 1-(2,3-dichlorophenyl)piperazine**

1-(2,3-Dichlorophenyl)piperazine.HCl was synthesized as previously reported26. To Boc-Gly/Pro-OH (0.002 mol) dissolved in acetonitrile (10 mL/g of compound) and cooled to 0°C was added NMM (0.21 mL, 0.002 mol). To this EDCI (0.383 g, 0.002 mol) dissolved in acetonitrile (4 mL) was added and stirred while maintaining the temperature at 0°C. After stirring the reaction mixture for 10 min at this temperature, HOBt (0.306 g, 0.002 mol) in DMF (3 mL) was added slowly. The reaction mixture was stirred for an additional 10 min and a pre-cooled solution of 2,3-dichlorophenyl piperazine.HCl (0.536 g, 0.002 mol) and NMM (0.21 mL, 0.002 mol) in DMF (5 mL) was added slowly. After 20 min the pH of the solution was adjusted to 8 by addition of NMM and the reaction mixture was stirred overnight at RT. Acetonitrile was removed under reduced pressure and the residual DMF was poured into about 100 mL ice-cold 90% saturated KHCO3 solution and stirred for 30 min. The precipitate was extracted into chloroform and washed sequentially with 5% NaHCO3 solution in DMF (5 mL) and then with 0.1N cold HCl (3 × 20 mL) followed by brine solution. The organic layer was dried over anhydrous Na2SO4, the solvent was removed under reduced pressure, triturated with ether and dried.

**Table I — Anti-inflammatory activities of synthesized urea and thiourea derivatives**

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Z</th>
<th>Anti-inflammatory activity IC50 (µg/mL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>H</td>
<td>O</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>1b</td>
<td>H</td>
<td>S</td>
<td>225 ± 1.85</td>
</tr>
<tr>
<td>1c</td>
<td>2F</td>
<td>O</td>
<td>125 ± 1.25</td>
</tr>
<tr>
<td>1d</td>
<td>2F</td>
<td>S</td>
<td>75 ± 0.98</td>
</tr>
<tr>
<td>1e</td>
<td>3F</td>
<td>O</td>
<td>215 ± 1.61</td>
</tr>
<tr>
<td>1f</td>
<td>4F</td>
<td>O</td>
<td>42 ± 1.11</td>
</tr>
<tr>
<td>1g</td>
<td>4F</td>
<td>S</td>
<td>30 ± 0.85</td>
</tr>
<tr>
<td>1h</td>
<td>3Cl</td>
<td>O</td>
<td>220 ± 1.10</td>
</tr>
<tr>
<td>1i</td>
<td>3Cl</td>
<td>S</td>
<td>215 ± 1.29</td>
</tr>
<tr>
<td>1j</td>
<td>4Cl</td>
<td>O</td>
<td>65 ± 0.98</td>
</tr>
<tr>
<td>1k</td>
<td>4Cl</td>
<td>S</td>
<td>60 ± 0.88</td>
</tr>
<tr>
<td>1l</td>
<td>2Br</td>
<td>S</td>
<td>225 ± 1.32</td>
</tr>
<tr>
<td>1m</td>
<td>3Br</td>
<td>O</td>
<td>285 ± 1.41</td>
</tr>
<tr>
<td>1n</td>
<td>3Br</td>
<td>S</td>
<td>185 ± 1.15</td>
</tr>
<tr>
<td>1o</td>
<td>4Br</td>
<td>O</td>
<td>65 ± 0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Indomethacin 58 ± 0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ibuprofen 67 ± 0.62</td>
</tr>
</tbody>
</table>

*Values are mean of three determinations, the range of which are < 5% of mean in all cases.
General procedure for the synthesis of ureido and thioureido derivatives (1a-1o/2a-2o)

Boc-Xaa-PZN (0.150 g) [where Xaa = Gly or Pro] was stirred with 1.5 mL of TFA for 45 min at RT. After completion of the reaction (monitored by TLC), TFA was removed under vacuum, triturated with dry ether, filtered, washed with ether and dried to obtain TFA.H-Xaa-PZN.

Further, TFA.H-Xaa-PZN (0.001 mol) was dissolved in DMF (10 mL/g of compound), cooled to 0°C and NMM (0.10 mL, 0.001 mol) was added. To this solution respective substituted phenyl isocyanates and isothiocyanates (0.0012 mol) was added dropwise while maintaining the temperature at 0°C. The reaction mixture was stirred for 8 h slowly warming to RT. DMF was evaporated under high vacuum and the residue was poured into about 50 mL ice-cold 90% saturated KHCO$_3$ solution and stirred for 30 min. The precipitate was extracted into chloroform and washed sequentially with 5% NaHCO$_3$ solution (2 × 10 mL), water (2 × 10 mL), 1N citric acid (2 × 10 mL) followed by brine solution. The organic layer was dried over anhydrous Na$_2$SO$_4$. The solvent was removed under reduced pressure, triturated with hexane and dried under vacuum. The analytic and spectroscopic data of all the synthesized compounds are in close agreement with our earlier data published.$^{20}$

Anti-inflammatory activity

Human erythrocyte suspension

The whole blood was collected from a healthy volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and collected in heparinzed vacutainer. The blood was washed three times with 0.9% saline and centrifuged simultaneously for 10 min at 3000 rpm. The packed cells were washed with 0.9% saline and 40% v/v suspension made using isotonic phosphate buffer which was composed of 154 mM NaCl in 10 mM sodium phosphate buffered solution alone. The standard drugs indomethacin and ibuprofen were treated similar to test concentration. The experiment was carried out in triplicate. The mixtures were incubated for 10 min at RT, centrifuged for 10 min at 3000 rpm and absorbance of the supernatant was measured spectrophotometrically at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated from the following equation.

$$\text{% Inhibition of haemolysis} = \left( \frac{A_1 - A_2}{A_1} \right) \times 100$$

Where:
- $A_1$ = Absorbance of hypotonic buffered solution alone
- $A_2$ = Absorbance of test /standard sample in hypotonic solution

Conclusions

In summary, novel anti-inflammatory agents were synthesized by conjugating 2,3-dichlorophenyl piperazine with glycine and proline and converting them into ureido and thioureido derivatives. The results indicated that halogen substituents on urea and thiourea moieties are essential to exhibit good anti-inflammatory properties. Further, it was noticed that compounds containing halogen at para substitutions on the phenyl ring showed excellent activity compared to compounds with ortho and meta substituted compounds. This confirms that variation of the position of the substituents also affected the anti-inflammatory activity profile.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

Acknowledgements

The authors gratefully acknowledge University Grants Commission (UGC), New Delhi for awarding UGC-Post Doctoral Fellowship (P DFS), BSR faculty fellowship and DST Inspire fellowship.

References