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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Bioisosteric synthesis of nitrogen containing derivatives of salicyl alcohol, their in vivo pharmacological studies with molecular modeling

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Abstract Starting with salicylaldehyde, compounds (**I**) [1-(2-hydroxybenzyl)piperidinium chloride] and (**II**) [4-carbamoyl-1-(2-hydroxybenzyl)piperidinium chloride] were prepared via multi step synthesis. The synthesized compounds were evaluated in vivo for their anti-inflammatory, analgesic, and anti-pyretic activities. Both compounds showed significant pharmacological profile when compared with reference standard, aspirin. In an attempt to understand the ligand–protein interaction in terms of the binding affinity, the synthetic molecule **II** was subjected to docking analysis using AutoDock which showed better binding modes with the active sites of COX's enzymes.

Keywords 1-(2-Hydroxybenzyl)piperidinium chloride · 4-Carbamoyl-1-(2-hydroxybenzyl)piperidinium chloride · Anti-inflammatory · Anti-pyretic · Analgesic · Molecular docking

Introduction

Pharmacological activities of drugs depend on their reception in the living system. In majority of cases, drugs work through complex mechanistic pathways which are controlled by many factors. It is mainly the chemical structure of a drug which determines its ultimate pharmacological responses. Some pharmacological activities are clinically desirable to treat a particular disease while others may be undesirable and may cause toxicity; so structural changes are required to minimize toxic effects and maximize health benefits. Salicin (Fig. 1), first reported by Machagan was used in the treatment of acute rheumatism and was obtained from willow bark (*Salix alba)* (Machagan, 1876). Salicin is a glycoside of saligenin (2-hydroxy methyl phenol; salicyl alcohol; Fig. 2), and is chemically related to aspirin and possesses almost comparable pharmacological activities. Salicin on hydrolysis results in liberation of saligenin. Phenolic hydroxyl group of saligenin is making a β-glucosidic bond with D-glucopyranose in salicin structure. We were interested in the replacement of methylene hydroxyl group with nitrogen to see desirable pharmacological activities. In general, it has been argued that exchanging one bioisostere for another enhances the biological and/or physical properties of a compound, without making significant changes in a chemical structure (Joseph *et al.*, 1999). Recently, it has been reported that salicin acts as anti-pyretic pro-drug with no propensity for gastric injury (Akao *et al*., 2002). Salicyl alcohol which is a part of salicin structure has also been reported to have local anesthetic properties (Harischfelder *et al*., 1920; Harischfelder and Wynne, 1920). Moreover, Willson and his co-workers have found anti-pyretic and anesthetic activities of saligenin (Charles and Tony, 1956). Lately, discovery of long acting saligenin adrenergic receptor agonists incorporating uracil ring and urea has been reported (Procopiou *et al*., 2011a, 2011b). It has also been reported that certain 4-hydroxypiperidine derivatives possess analgesic activities (Saeed *et al*., 1997). The great

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importance of saligenin as a drug or pro-drug and piperidine derivatives possessing analgesic activities encouraged us to combine these together as bioisosters and evaluate their activities. Piperidinium and piperidinium-4-carboxamide derivatives were prepared for evaluation.

Non-steroidal anti-inflammatory drugs (NSAIDs) are used worldwide for the management of pain, inflammation, and hyperpyrexia while opiates are prescribed for severe pain that cannot be controlled by classical NSAIDs. However, across the globe, it is still a major challenge to the medical world that on chronic administration, NSAIDs exhibit several side effects like ulceration and subsequent bleeding of gastrointestinal tract, cardiovascular disorders, and renal damage while opiates cause dependence and drug tolerance, which limits their therapeutic spectrum value (Pérez *et al*., 1996; Jones *et al*., 2008; Mayer *et al*., 1995). Therefore, there is a definite need for an alternative to opiates and NSAIDs which possess analgesic, anti-inflammatory, and anti-pyretic activities yet devoid of such adverse effects. The current work was a step forward in this direction.

We synthesized salicyl alcohol derivatives by incorporating piperidine and piperdine-4-carboxamide moieties. These were evaluated for their analgesic, anti-inflammatory, and anti-pyretic pharmacological profiles. The interaction of the synthesized compounds with binding sites of COX's was examined using molecular modeling to rationalize their activities. The structures of the products were confirmed by physical data and spectroscopic methods like (IR, $^{-1}$H and ^{13}C NMR, and mass spectrometry). Furthermore, due to their solubility in water as compared to aspirin, these may be used for parental and oral liquid dosage forms.

Methods and materials

Chemistry

General

Melting points of the compounds were determined with Gallenkamp Melting Point apparatus. Infrared spectra were recorded on Nicolet 380 thermoscientific FTIR. NMR spectra were scanned on BRUKER 300-MHz spectrophotometer using D_2O as solvent. The chemical shifts were reported as parts per million (δ ppm) using TMS as an internal standard. Mass spectra were made on JEOL MSRoute spectrometer. Digital Plethysmometer (Model LE 7500 Plan lab S.L.) was used to measure the paw volume and digital thermometer (Model CA92121, ACON Laboratories, USA) was used to measure the rectal temperature.

Fig. 1 Salicin

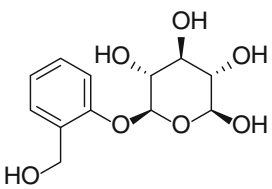
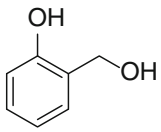


Fig. 2 Salicyl alcohol



Glacial acetic acid (Panreac, Spain), acetylsalicylic acid (Sigma, USA), Lambda Carrageenan (Sigma, USA), Brewer yeast (Merck, Germany), morphine sulfate were obtained through proper channel from Punjab Drug House, Lahore, Pakistan. Salicylaldehyde was purchased from Sigma-Aldrich. All the chemicals were used without further purification.

Synthesis

Starting from salicylaldehyde, compounds **I** and **II** were synthesized by the following general procedure;

A mixture of salicylaldehyde (10 mmol), $NaBH_4$ (10 mmol), and boric acid (10 mmol) were pulverized with mortar and pestle until TLC showed complete disappearance of the starting material. The reaction mixture was quenched with 1 N HCl (10 mL) solution and extracted with ethyl acetate. The ethyl acetate layer was separated and dried over sodium sulfate. On evaporation of the solvent, pure salicyl alcohol was obtained, which was used in further reactions without purification.

To a solution of salicyl alcohol (1 mmol) in dichloromethane, thionyl chloride was slowly added at room temperature. When the addition was complete, the reaction mixture was refluxed for 2 h. It was cooled to 0 °C and amine (1 mmol) was slowly added. The product precipitated as white powder. It was filtered, washed with dichloromethane, and kept under vacuum for 24 h (Fig. 3).

1-(2-Hydroxybenzyl)piperidinium chloride (***I***) Yield, 65.5 %; mp 160 °C; IR (neat): υ cm^{-1} 3381, 2947, 2731, 1591, 1456, 1136; ^{1}H NMR (300 MHz, D_2O): δ 7.45–13 (m, 4H), 4.55 (s, 2H), 3.08 (t, 4H), 1.71 (m, 4H), 1.63 (m, 2H); ^{13}C NMR (75 MHz, D_2O): δ 158.5, 137.5, 134.9, 123.0, 121.6, 119.0, 58.7, 47.2 (2C), 24.8 (2C), 24.1; ESI–MS (*m*/*z*): M+191

4-Carbamoyl-1-(2-hydroxybenzyl)piperidinium chloride (***II***) Yield, 58.7 %; mp 206 °C (decomposes); IR (neat): υ cm^{-1} 3387, 3203, 3001, 2937, 2817, 1667, 1619, 1429, 610; ^{1}H NMR (300 MHz, D_2O): δ 7.39–7.01 (m, 4H), 4.12 (s, 2H),

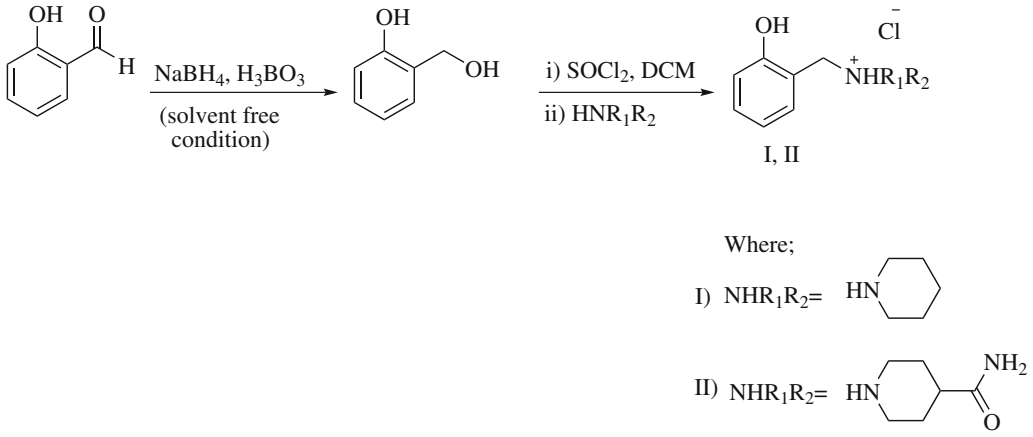


Fig. 3 Synthesis of compounds **I** and **II**

3.05 (m, 4H), 2.66 (m, 1H), 1.87 (m, 4H); ^{13}C NMR (75 MHz, D_2O): 181.8, 158.2, 135.6, 134.7, 130.7, 123.2, 118.5, 58.5, 45.8 (2C), 41.8, 27.6 (2C); ESI–MS (*m/z*): M+234

Pharmacology

Animals

For pharmacological study, Albino mice (*Balb-C*), weighing 25–30 g of either sex, were used. For each pharmacological assay, animals were randomly divided into four groups and each group was composed of eight animals.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenan-induced paw edema test (Winter *et al*., 1962). Aspirin was administered as a standard drug for comparison. The test compounds were administered at three dose levels (50, 100, and 150 mg kg^{-1}). The paw volumes were measured using a digital Plethysmometer immediately before and 1, 2, 3, 4, and 5 h after carrageenan injection. The percentage inhibition of paw edema was calculated using the following formula:

$$\% \text{ Inhibition} = 100\,[1 - (a - x)/(b - y)],$$

where x is the mean paw volume of mice before the administration of carrageenan and test compounds or reference compound (test group), a is the mean paw volume of mice after the administration of carrageenan in the test group (drug treated), b is the mean paw volume of mice after the administration of carrageenan in the control group, and y is the mean paw volume of mice before the administration of carrageenan in the control group.

Anti-pyretic study

Hyperpyrexia was induced by subcutaneous injection of 20 % solution of Brewer's yeast (Al-Ghamdi, 2001; Al-Harbi *et al*., 1994). Changes in rectal temperature were noted after 24 h of Brewer's yeast injection at 0.5, 1.0, and 1.5 h. Before insertion, the rectal probe of digital thermometer was lubricated with olive oil and maintained for 30-s insertion time for recording temperature.

Analgesic activity

Acetic acid (AA)-induced writhing test in mice Writhing behavior was induced by intraperitoneal administration of 1 % AA (10 mL kg^{-1}). The number of writhes (abdominal constrictions) occurring over a period of 20 min were counted (starting 5 min after the administration of 1 % AA) (Gray *et al*., 1999, 1998).

$$\% \text{ Protection} = (1 - \text{mean number of writhes of treated drug/mean number of writhes of control}) \times 100.$$

Hot plate test in mice Prior to the start of experimental procedure, mice were acclimatized to laboratory environment at least for 2 h. A transparent glass cylinder was used to restrict the animals to the surface of hot plate of analgesiometer (Harvard apparatus, USA) and the temperature maintained at 54.0 ± 0.10 °C. Hot plate reaction time (latency to response in seconds) was observed by noting licking, flicking of hind limb, or jumping from cylinder (Brochet *et al*., 1986; Grillet *et al*., 2005). A cut-off time of 30 s was fixed so that if the animal did not respond within the prescribed time, then they could be immediately removed from the hot plate surface to avoid tissue damage. 30 min after the pretest, control, standard, and test samples were distributed into their respective groups. The animals were tested again after 30 min and response recorded at 30

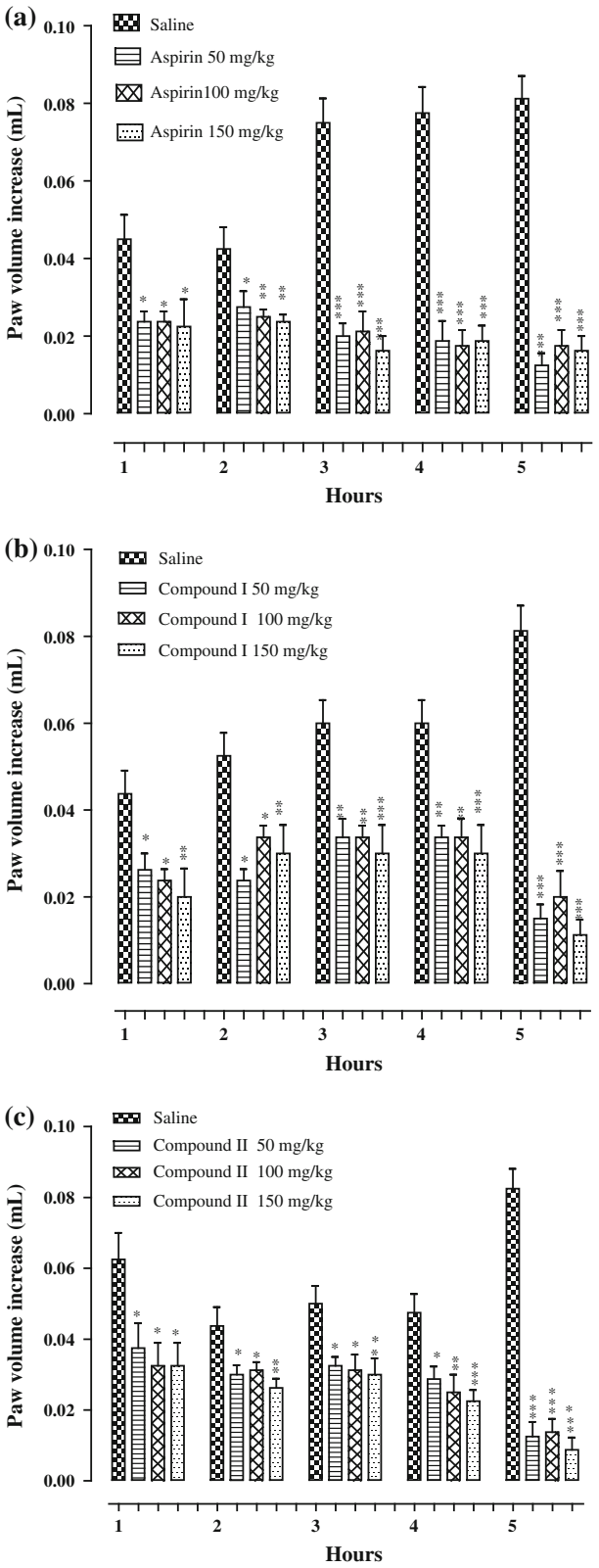


Fig. 4 **a** Anti-inflammatory activity of aspirin in Carrageenan-induced Paw edema model. **b** Anti-inflammatory activity of compound **I** in Carrageenan-induced Paw edema model. **c** Anti-inflammatory activity of compound **II** in Carrageenan-induced Paw edema model

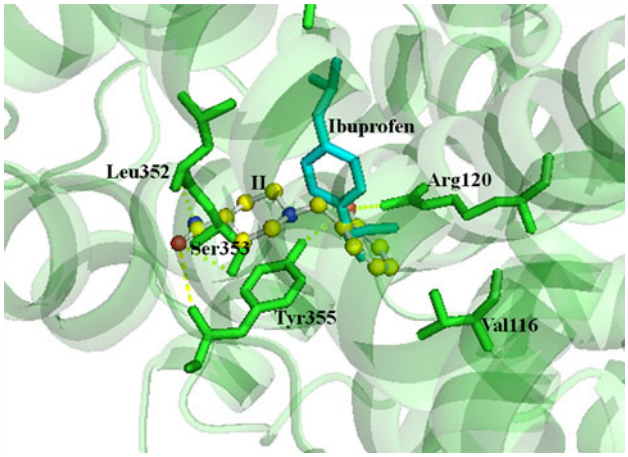


Fig. 5 Binding mode of compound **II** in active site of COX-1 (PDB Code: 1EQG). *Colors* of atoms are as follow: *yellow* carbon, *blue* nitrogen, and *red* oxygen. The native co-crystallized ibuprofen is shown as *stick* (*cyan*) and hydrogen bonds are shown as *dashed lines*

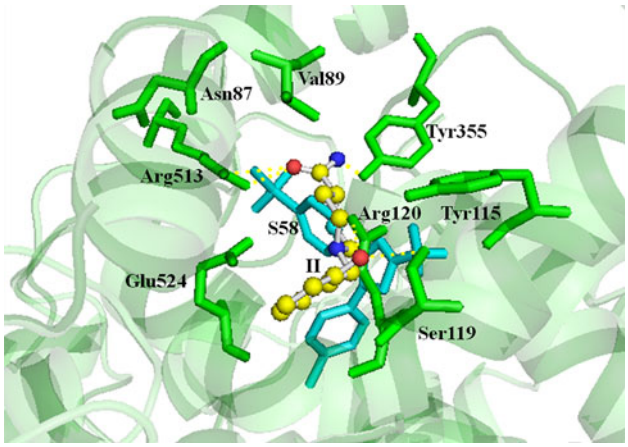


Fig. 6 Binding mode of compound **II** in active site of COX-2 (PDB Code: 1CX2). *Colors* of atoms are as follow: *yellow* carbon, *blue* nitrogen, and *red* oxygen. The native co-crystallized S58 is shown as *stick* (*cyan*) and hydrogen bonds are shown as *dashed lines*

and 60 min on the hot plate of analgesia meter. Analgesic activity was calculated using the following formula:

$$\% \text{ Analgesic activity} = (\text{Test latency} - \text{control latency}) / (\text{Cut - off time} - \text{control latency}) \times 100$$

Statistical analysis

Statistical analysis of the biological activity of the synthesized compounds on animals was evaluated using a one-way analysis of variance (ANOVA). In all cases, post hoc comparisons of the mean of individual groups were performed using Dunnett's test. A significance level of

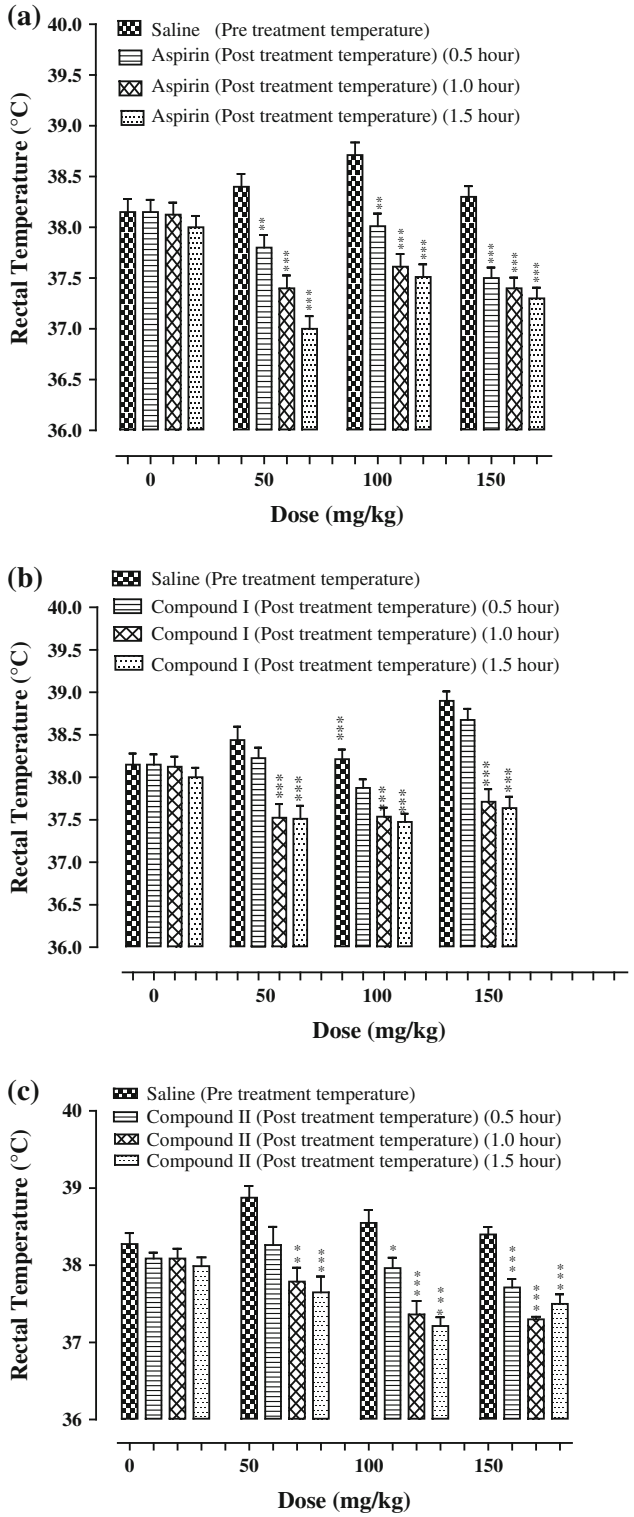


Fig. 7 **a** Anti-pyretic activity of aspirin in Brewer's yeast-induced pyrexia test. **b** Anti-pyretic activity of compound **I** in Brewer's yeast-induced pyrexia test. **c** Anti-pyretic activity of compound **II** in Brewer's yeast-induced pyrexia test

$p < 0.05$ denoted significance in all cases. All values are expressed as mean ±SD (standard deviations). For statistical analysis we have used Graph Pad Prism 5.0 version.

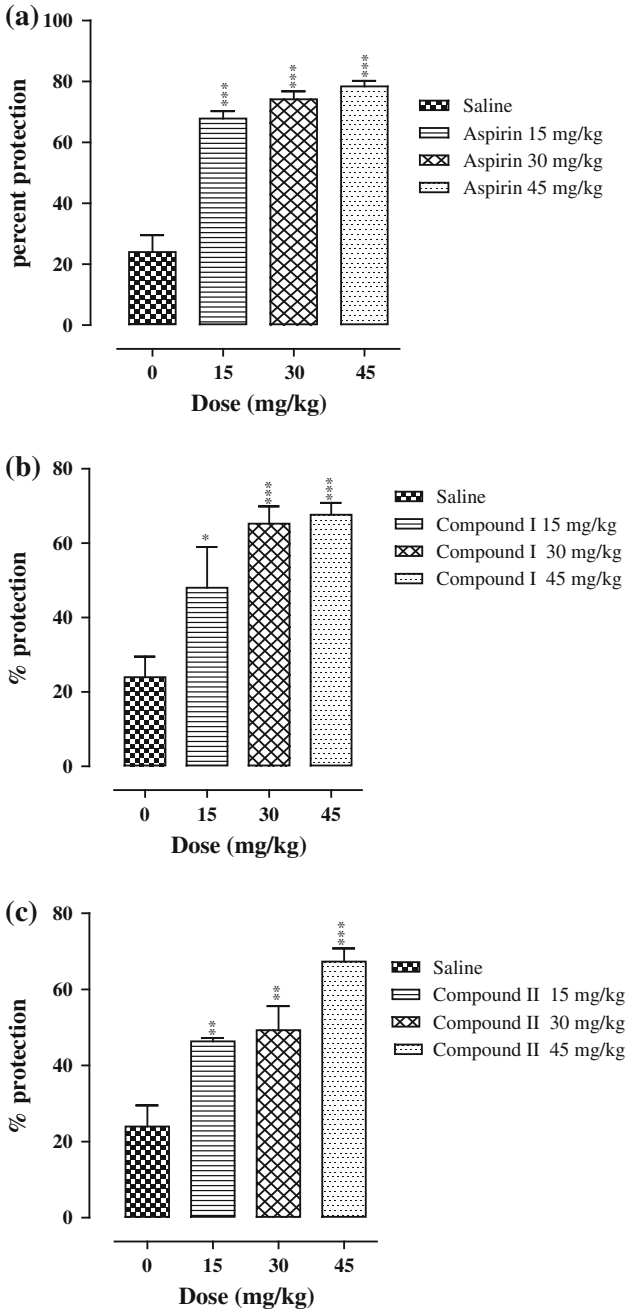


Fig. 8 **a** Anti-nociceptive activity of aspirin in writhing test. **b** Anti-nociceptive activity of compound **I** in writhing test. **c** Anti-nociceptive activity of compound **II** in writhing test

Molecular docking

The COX-1 (PDB Code: 1EQG) and COX-2 (PDB Code: 1CX2) crystal structures were used for docking. AutoDock tools were used to remove water molecules from the crystal structures of COXs. The polar hydrogens were added and charges were assigned with the Gasteiger method. Then, the active sites were defined by fixing the grid box having dimensions 40 × 40 × 40 Å with grid spacing of 0.375 Å

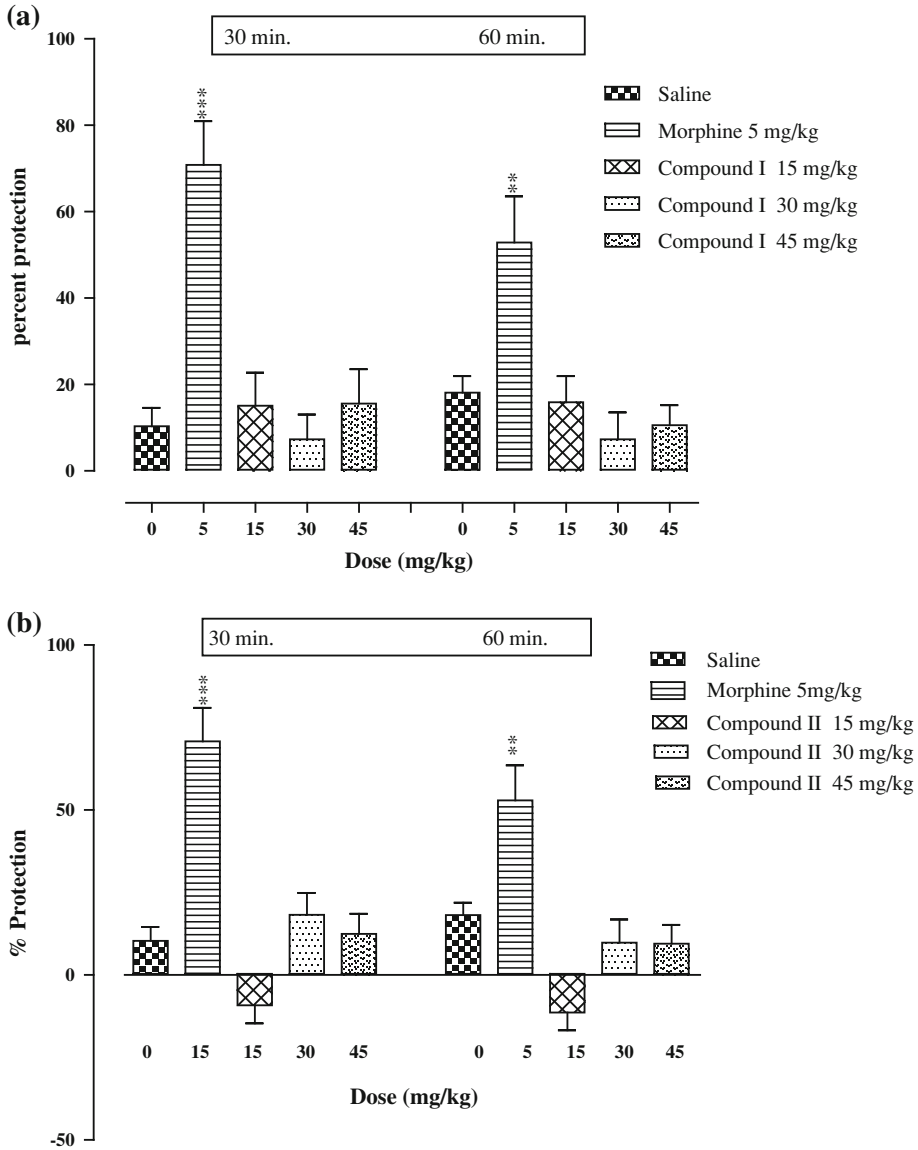


Fig. 9 **a** Anti-nociceptive activity of compound **I** in hot plate mode. **b** Anti-nociceptive activity of compound **II** in hot plate model

centered on the active site of the protein, and the structures of the enzymes as receptors into the required pdbqt formats were saved. To gain insight into the possible binding modes, compound **II** in the active site of the COX's enzymes was examined using AutoDock Vina (Trott and Olson, 2010).

Results and discussion

Anti-inflammatory activity was evaluated by the carrageenan-induced paw edema test in mice. The anti-inflammatory activity data (Fig. 4a–c) indicated that compounds **I** and **II** protected mice from carrageenan-induced inflammation moderately at 1-h reaction time, with activity increasing to a peak level at 5 h. Compound **II** [4-carbamoyl-1-(2-hydroxybenzyl)piperidinium chloride] exhibited the most potent anti-inflammatory activity and it is moderately more potent when compared with the reference standard aspirin.

The interaction of compound **II** with binding sites of COXs was examined using molecular modeling to rationalize their activities. The lowest energy binding orientation of compound **II** in the active site of COX-1 shown in Fig. 5 indicates that the hydroxyl group of inhibitor (compound **II**) is involved in the polar interactions, which include the hydrogen bonds between the inhibitor and amino group of Arg120 (3.0 Å) and phenolic hydroxyl of Tyr355 (3.0 Å). Besides these polar interactions, the oxygen atom of the amide moiety of the inhibitor makes hydrogen bonds with amino group of Tyr355 (3.1 Å) and hydroxyl of Ser353

(3.1 Å), while the nitrogen atom of amide moiety interacts with Leu352, which firmly locks the inhibitor in the active site of COX-1.

The molecular modeling of compound **II** in the active site of COX-2 enzyme (Fig. 6) shows that compound **II** almost superimposed on the native ligand (S58) and exhibits one strong hydrogen bond between C–NH_2 of inhibitor and *p*-OH of Tyr355 (1.4 Å), while C=O moiety of the inhibitor **II** has two hydrogen bonds with Arg513 (3.1 Å and 3.2 Å). The hydroxyl group of the inhibitor bounds to Ser119 (3.1 Å) and Arg120 (3.1 Å) amino acids of the COX-2 enzyme.

The synthesized compounds **I** and **II** were screened for anti-pyretic activity using the Brewer's yeast-induced hyperpyrexia method. The graphical results (Fig. 7a–c) show that compounds **I** and **II** significantly decreased the temperature of pyretic mice at 0.5, 1.0, and 1.5 h after intraperitoneal (i.p) administration of the compounds. The maximum mean rectal temperatures produced by Brewer's yeast in the presence of compounds **I** and **II** were found to be 37.7 and 37.2 °C, respectively, after 3 h, compared to the control group 38.7 °C.

The analgesic activities of the compounds were studied by AA-induced writhing test in mice. The analgesic activity was evaluated at doses equivalent to 15, 30, and 45 mg kg^{-1} (aspirin).These compounds presented an important analgesic profile measured by the classical AA-induced writhing model. From the results of AA-induced writhing test, it was noticed that compounds **I** and **II** possess significant analgesic activity (Fig. 8a–c). The analgesic effects of **I** and **II** (67.62 and 67.34 %, respectively) were found to be comparable to that of the reference standard aspirin (78.38 %).

As depicted in Fig. 9a and b, morphine used as a standard at a dose level of 5 mg kg^{-1} significantly increases latency time on hot plate, while compounds **I** and **II** failed to increase latency time significantly. ANOVA followed by Dunnett's post hoc analysis of morphine revealed dose-dependent increase in latency (** $p < 0.01$, *** $p < 0.001$), while compounds **I** and **II** failed to increase latency time in the hot plate test.

Conclusions

In this study, we have reported the synthesis of one new and one reported derivatives of salicyl alcohol. The results of analgesic, anti-inflammatory, and anti-pyretic screening showed that both the compounds (**I** and **II**) possess enhanced activity compared to the reference standard aspirin. 4-Carbamoyl-1-(2-hydroxybenzyl)piperidinium chloride (**II**) emerged as the most active compound in exhibiting analgesic, anti-pyretic, and anti-inflammatory activity when compared with the reference standard aspirin. Hence, this series could be developed as a novel class of analgesic, anti-pyretic, and anti-inflammatory agents. However, further structural modifications are needed to increase the analgesic, anti-pyretic, and anti-inflammatory activities with decreased ulcerogenicity.

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References

Akao T, Yoshino T, Kobashi K, Hattori H (2002) Evaluation of salicin as an antipyretic prodrug that does not cause gastric injury. Planta Med 68:714–718

Al-Ghamdi M (2001) The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. J Ethnopharmacol 76:45–48

Al-Harbi, Quereshi S, Ahmed M, Raza M, Miana G, Shah A (1994) Studies on the antiinflammatory, antipyretic and analgesic activities of santonin. Jpn J Pharmacol 64:135–139

Brochet D, Micó JA, Martin P, Simon P (1986) Antinociceptive activity of beta-adrenoceptor agonists in the hot plate test in mice. Psychopharmacology 88:527–528

Charles O, Tony E (1956) The American drug index. J. B Lippincott Co, Philadelphia

Gray A, Spencer P, Sewell R (1998) The involvement of the opioidergic system in the antinociceptive mechanism of action of antidepressant compounds. Br J Pharmacol 124:669–674

Gray A, Nevinson M, Sewell R (1999) The involvement of opioidergic and noradrenergic mechanisms in nefopam antinociception. Eur J Pharmacol 365:149–157

Grillet N, Pattyn A, Contet C (2005) Generation and characterization of Rgs4 mutant mice. Mol Cell Biol 25:4221–4228

Harischfelder A, Wynne H (1920) Saligenin as a local anesthetic for the female urethra. JAMA 75:1770

Harischfelder A, Lundholm A, Norrgard H (1920) The local anaesthetic actions of saligenin and other phenyl carbinols. J Pharmacol Exp Ther 15:227–261

Jones R, Rubin G, Berenbaum F, Scheiman J (2008) Gastrointestinal and cardiovascular risks of nonsteroidal anti-inflammatory drugs. Am J Med 121:464–474

Joseph B, Danuta M, Arthi K, Virginia L, David L, David E (1999) Thieno[3,2-b]- and thieno[2,3-b]pyrrole bioisosteric analogues of the hallucinogen and serotonin agonist *N*,*N*-dimethyltryptamine. J Med Chem 42:1106–1111

Machagan T (1876) The treatment of acute rheumatism by salicin. Lancet 1:342–343

Mayer D, Mao J, Price D (1995) The development of morphine tolerance and dependence is associated with translocation of protein kinase C. Pain 61:365–374

Pérez G, Duque O, Raiford D, García R, Ris R (1996) Nonsteroidal anti-inflammatory drugs and the risk of hospitalization for acute renal failure. Arch Intern Med 156:2433

Procopiou P, Barrett V, Ford A, Looker B, Lunniss G, Needham D, Smith C, Somers G (2011a) The discovery of long-acting saligenin β_2 adrenergic receptor agonists incorporating hydantoin or uracil rings. Bioorg Med Chem 19:4192–4201

Procopiou P, Barrett V, Ford A, Looker B, Lunniss G, Needham D, Smith C, Somers G (2011b) The discovery of long acting β adrenergic receptor agonists incorporating a urea group. Bioorg Med Chem 19:6026–6032

Saeed M, Saify Z, Zafar I, Islam Nazar-ul (1997) Synthesis of 4-hydroxypiperidine derivatives and their screening for analgesic activity. Arch Pharm Res 20:338–341

Trott O, Olson A (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 31:455–461

Winter C, Risley E, Nuss G (1962) Carrageenan induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proc Soc Exp Biol Med 111:544–547