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Synthesis and Anti-Inflammatory Testing of Some New Compounds Incorporating 5-Aminosalicylic Acid (5-ASA) as Potential Prodrugs*

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This work includes the synthesis of 15 final compounds (**6a-h** and **7b-h**) as prodrugs of 5-ASA in the form of the acid itself, esters and amides linked by an amide linkage through a spacer to the endocyclic ring N of nicotinamide. Also, 15 new intermediate compounds were prepared. The target compounds (**6b**, **6f**, **7b**, and **7e-h**) revealed potent analgesic and anti-inflammatory activities in comparison to sulfasalazine and 5-ASA. In addition, ulcerogenicity, LD_{50}, *in vivo* and *in vitro* metabolism of compound **7f** were determined.

Key words: Prodrugs, 5-ASA, SASP, Nicotinamide, Analgesic, Anti-inflammatory, Ulcerative colitis

INTRODUCTION

5-ASA (Thomson *et al.*, 2000; Orlikovs, 2001) and some of its derivatives (Shen *et al.*, 1972; Jones *et al.*, 1978; Möller *et al.*, 1989) have been tested as anti-inflammatories, analgesics and proved higher therapeutic index with less or no gastric toxicity in comparison to NSAD'S. Derivatives of 5-ASA are also recommended as anti-inflammatories in severe dermatological disorders including psoriasis and other dermatitis (Jorgensen *et al.*, 1989; Margaretha *et al.*, 1990).

Colon- specific prodrugs aiming at the delivery of 5-ASA to the colon have been introduced. The earliest-accepted formulation was sulfasalazine (SASP) an azo-conjugate of 5-ASA with sulfapyridine (SP). With the knowledge that the adverse effects associated with SASP are due to SP (Brown *et al.*, 1983).

Therefore, SP was replaced by 4-aminobenzoyl-β-alanine in balsalazide (Chan *et al.*, 1983) and by another 5-ASA molecule in olsalazine (Willoughby *et al.*, 1982). Non azo-conjugate of 5-ASA, including 5-ASA-glycine (Jung *et al.*, 2000) and 5-ASA-*O*-sulfate ester (Rokos *et al.*, 1988) were also developed. Polymeric prodrugs (Brown *et al.*, 1983; Davaran *et al.*, 1999) with 5-ASA linked to the polymer backbones *via* azo, ester and amide bonds, are susceptible to enzymatic attack in the large intestine and 5-ASA is released at this site.

In view of these data, the present work aims at the synthesis of 5-ASA amides and esters linked through a spacer to nicotinamide by an amide bond (**6a-h** and 7**b-h**, Scheme 1). The following considerations were, also, taken into account as guidelines in designing the proposed compounds:

1- It has been reported that the fifth position of salicylic acid is the most favourable position for substitution to enhance the anti-inflammatory activity of salicylic acid while decreasing toxicity (Jones *et al.*, 1978).

2- Salicylic acid salt of nicotinamide was found to have better water solubility, non irritability, good tolerability, excellent anti-inflammatory, analgesic and antipyretic actions along with low toxicity (Hans *et al.*, 1985).

3- Nicotinamide is used as a solubilizing agent for the poorly water-soluble drugs (Truelove *et al.*, 1984).

4- 1,4-Dihydronicotinamide is used as safe chemical delivery system to deliver the water-soluble compounds to the brain and other body compartments (Bodor *et al.*, 1984, 1985; Phelan *et al.*, 1989). Also, it is a component of vitamin B complex along with its importance for the biosynthesis of NAD ⇌ NADH redox system *in vivo*.

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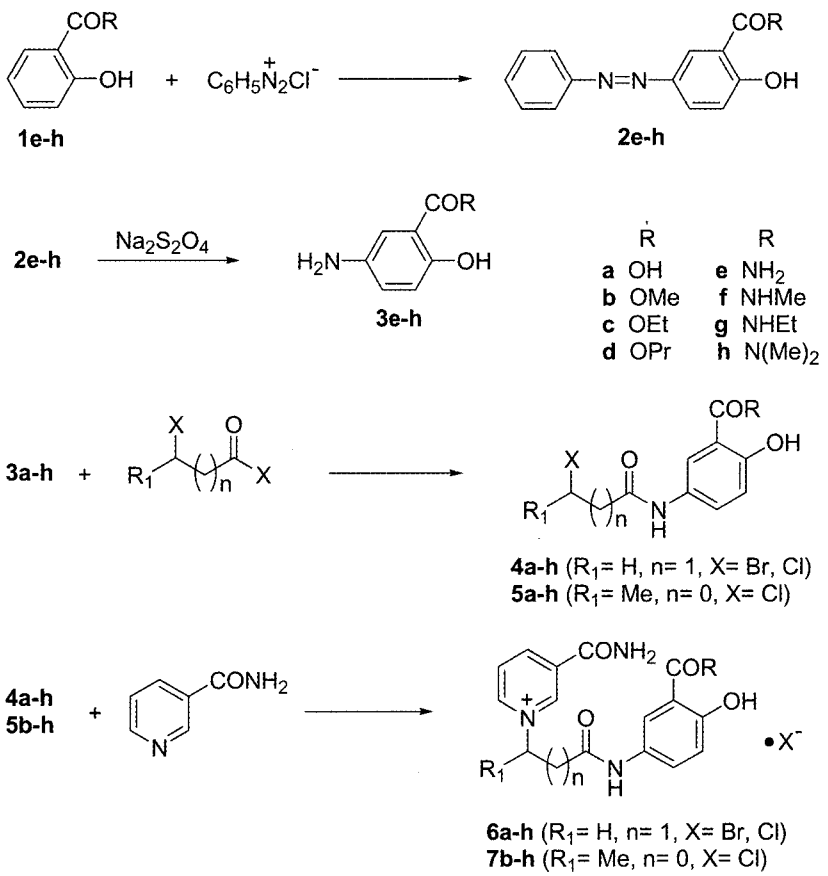

Scheme 1. Synthesis of the target compounds

MATERIALS AND METHODS

Melting points were determined on an electrothermal melting point apparatus and are uncorrected.

Precoated silica gel plates 60 F-254 plates from Merck were used for TLC monitoring of reactions; spots were detected under UV lamb (MODEL CM-10, U.S.A.).

Infrared spectra were recorded on Shimadzu 200-91527 spectrophotometer. All compounds were done in KBr disks. ^{1}H-NMR spectra were run on an EM-360 varian 60 MHz NMR spectrometer using DMSO-d_6 as solvent and TMS as an internal standard (chemical shifts in δ ppm) at the faculty of pharmacy, university of Assiut.

EI (70 ev) and FAB mass spectra were performed with JEOL JMS 600 at the central laboratory, university of Assiut. Elemental analyses were done on a Perkin-Elmer 240 elemental analyzer at the central laboratory, university of Assiut.

Salicylamides **1e-h** (Jensen *et al.*, 1949; Coates *et al.*, 1957), methyl, ethyl and propyl 5-aminosalicylates (**3b-3d**) (Jorgensen *et al.*, 1990) were prepared according to reported procedures. The physical and spectral data of the n-propyl ester (table VII) are not found in the available literatures, while those of the methyl and ethyl esters are compatible with the reported (Jorgensen *et al.*, 1990).

SASP (Pharmacia) was donated by Kahira Pharm. Chem. Industrial Co., Cairo, Egypt. 5-ASA (Fluka), acetylsalicylic acid (El-Naser CO.) and other chemicals and solvents were obtained from the local market.

Adult male albino mice and rats were obtained from animal house of the faculty of Medicine, Assiut University. Male Sprague-Dawely rats were obtained from the animal house of the National Organization for Bioproducts and Vaccines in Helwan.

Streptococcus pyogenus cultivated on blood agar medium was obtained from Department of Microbiology, Faculty of Medicine, El Minia University.

Preparation of 5-aminosalicylamides (3e-h)

Preparation of the azo dyes (2e-h)

To an ice cooled solution of aniline (12.5 mL, 0.138 mole) in concentrated hydrochloric acid (40 mL), a solution of sodium nitrite (10 g, 0.145 mole) in water (20 mL) was added, slowly, with stirring, while keeping the temperature below 5°C. The obtained diazonium salt solution was added slowly with stirring to the cooled alkaline solution of the appropriate salicylamide (**1e-h**,

0.14 mole) in sodium hydroxide (10%, 90 mL). The mixture was further stirred for half an hour below 5°C, allowed to stand for three hours at the ambient temperature and the obtained azo dye (**2e-h**) was filtered, washed with water, dried and used in the next step without any purification.

Reduction of azo dyes (2e-h)

The appropriate azo dye (2e-h) was dissolved in NaOH solution (10%, 300 mL). The solution was heated and maitained at 80 °C, sodium dithionite (60 g) were added portionwise with stirring till the red colour of the solution fades and a thin layer of aniline collects on the surface. The reaction mixture was cooled and neutralized with concentrated hydrochloric acid, where a yellowish white precipitate of the appropriate 5-aminosalicylamide (**3e-h**) was obtained. The precipitate was filtered, washed with water and crystallized from water. Yields, m.p. and spectral data are recorded in Table I.

Preparation of 5-(haloacylamino)salicylic acid esters and amides (4a-h and 5a-h)

The appropriate haloacylhalide R^1-$CHX(CH_2)_nCOX$ (0.02 mole) in dry benzene (5 mL) was added dropwise to a cooled suspension of 5-aminosalicylic acid, its appropriate ester or amide (**3a-h**, 0.02 mole) in dry benzene (50 mL) while stirring. The mixture was refluxed for 24 h until evolution of hydrogen chloride gas was ceased. The reaction mixture was evaporated under reduced pressure, where a solid residue was obtained; the residue was filtered, washed with cooled water, hydrochloric acid (2%), again with cooled water, dried and crystallized from aqueous ethanol (50%). Physical and spectral data are shown in Tables II and III.

Preparation of 3-carbamoyl-1-(*N*-(4'-hydroxy-3'-substituted phenyl)carbamoyl)alkylpyridinium halides (6a-h and 7b-h)

Nicotinamide (0.002 mole) and the appropriate haloacylated-5-aminosalicylic acid ester or amide (**4a-h** and **5b-h**, 0.002 mole) in acetonitrile (15 mL) were refluxed for 7-10 days until sufficient products have been formed as monitored by TLC using chloroform and methanol (6:1). The precipitate was then filtered, washed with acetonitrile and crystallized from the suitable solvent. Physical and microanalytical data are presented in Tables IV-VII.

Anti-inflammatory activity (Winter *et al.*, 1962)

Male adult albino rats (180-200 g), were divided into groups, each of five animals. Solutions or suspensions of the test compounds and reference drugs in 5% gum acacia were injected intraperitoneally (i.p.) into rats at a dose level of 10 mg/kg. One group of animals was used for each treatment. Control animals were similarly treated with 5% gum acacia. After 30 minutes, 0.2 mL of 1% carrageenin solution in normal saline was injected subcutaneously (s.c.) into the plantar surface of the right hind paw of all rats. The right paw volume was measured by a Veriner caliper (SMIEC) directly before and 1/2, 1, 2, 3, and 5 hours after carrageenin administration.

Results of the anti-inflammatory evaluation of the test compounds and reference drugs are listed in Table VIII.

Analgesic activity (Eddy *et al.*, 1953)

Male adult albino mice (20-28 g), were divided into groups, each of five animals. Solutions or suspensions of the test compounds and the reference drug in 5% gum acacia were injected i.p. in a dose level of 10 mg/kg into mice. Control animals were similarly treated with 5% gum

Table I. Physical and spectral data of 5-aminosalicylic acid derivatives (**3d-h**)

Compounds	Yield %	m.p. °C	^{1}H-NMR (DMSO-d_6, δ ppm)*
3d	50	Liquid**	0.95 (3H, t, $OCH_2CH_2C\underline{H_3}$); 1.36-2.06 (2H, m, $CH_2C\underline{H_2}CH_3$); 3.85-4.55 (4H, m, $C\underline{H_2}CH_2CH_2$ & NH_2); 6.55-6.90 (2H, m, C3-H & C4-H); 7.00 (1H, d, C6-H); 9.85 (1H, br. s, OH).
3e	58	186-188	6.63-7.86 (7H, m, 3 aromatic protons, NH_2 & $CONH_2$); 12.55 (1H, s, OH).
3f	62	146-148	3.05-4.85 (5H, m, CH_3 & NH_2); 6.70 (2H, dd, C3-H & C4 -H); 7.00 (1H, d, C6-H); 7.29-8.30 (2H, br. s, NH & OH).
3g	60	118-120	1.25 (3H, t, $CH_2C\underline{H_3}$); 3.15-3.70 (2H, m, $NHC\underline{H_2}CH_3$); 4.45 (2H, br. s, NH_2); 6.90 (2H, dd, C3-H & C4-H); 7.10 (1H, d, C6-H); 8.75 (1H, t, NH); 11.9 (1H, br. s, OH).
3h	65	158-160	2.95 (6H, s, $N(CH_3)_2$); 4.65 (2H, br. s, NH_2); 6.5 (1H, s, C3-H); 6.75 (2H, s, C4-H & C6-H); 8.85 (1H, br. s, OH).

*Protons of CONH and OH groups are exchangeable by D_2O.

**The liquid was purified by column chromatography (chloroform/methanol; 9:1).

The IR spectra (KBr, cm^{-1}) showed stretching bands at about:

3d: 3365 (OH), 3225 (NH_2, br.) and 1671 (C=O; ester)

3e-h: 4485 (OH), 3225 (NH) and 1640 (C=O; amide).

Table II. Physical and spectral data of 5-(β-halopropionylamino) salicylic acid esters and amides (**4a-h**)

Compounds	Yield %	m.p. °C	^{1}H-NMR (DMSO-d_6, δ ppm)*
4a	73	204-205	2.86 (2H, t, $COCH_2CH_2Br$); 3.66 (2H, t, $COCH_2CH_2Br$); 7.10 (1H, d, , J = 8.50 Hz, C3-H); 7.86 (1H, dd, C4-H); 8.33 (1H, d, C6-H); 10.26 (3H, br. s, NH, OH & COOH).
4b	75	123-124	2.90 (2H, t, CH_2CH_2Br); 3.70 (2H, t, CH_2CH_2Br); 3.88 (3H, s, OCH_3); 6.95 (1H, d, J = 8.50 Hz, C3-H); 7.50 (1H, dd, J = 8.50 Hz, C4-H); 7.85-8.15 (2H, m, C6-H & NH); 10.65 (1H, s, OH).
4c	67	113-114	1.40 (3H, t, CH_2CH_3); 2.86 (2H, t, CH_2CH_2Br); 3.66 (2H, t, CH_2CH_2Br); 4.40 (2H, q, OCH_2CH_3); 6.95 (1H, d, J = 8.50 Hz, C3-H); 7.23-7.65 (2H, br. dd, J = 8.50 Hz, NH & C4-H); 8.00 (1H, d, C6-H); 10.75 (1H, s, OH).
4d	65	108-110	1.00 (3H, t, $CH_2CH_2CH_3$); 1.50-2.13 (2H, m, $CH_2CH_2CH_3$); 2.93 (2H, t, $COCH_2CH_2$); 3.70 (2H, t CH_2CH_2Br); 4.35 (2H, t, $OCH_2CH_2CH_3$); 6.93 (1H, d, J = 8.50 Hz , C3-H); 7.55 (1H, dd, J = 8.50 Hz, C4-H); 7.82-8.20 (2H, br. d, NH & C6-H); 10.75 (1H, s, OH).
4e	70	189-190	2.90 (2H, t, $COCH_2CH_2$); 3.70 (2H, t, CH_2CH_2Br); 6.83 (1H, d, J = 8.50 Hz, C3-H); 7.20-8.20 (4H, m, C4-H, C6-H & $CONH_2$); 9.76 (2H, br. s, NH & OH).
4f	67	170-172	2.70-3.16 (5H, m, $COCH_2CH_2$ & $NHCH_3$); 3.70 (2H, t, CH_2CH_2Br); 6.86 (1H, d, J = 8.50 Hz, C3-H); 7.46 (1H, dd, J = 8.50 Hz, C4-H); 8.05 (1H, d, C6-H); 8.20 (1H, q, $NHCH_3$); 9.60 (2H, br. s, NH & OH)
4g	72	154-156	1.25 (3H, t, CH_2CH_3); 2.15-4.15 (6H, m, $COCH_2CH_2$, $NHCH_2CH_3$ & CH_2CH_2Br); 7.05 (1H, d, J = 8.50 Hz, C3-H); 7.45-7.80 (2H, m, C4-H & $NHCH_2CH_3$); 8.30 (1H, d, C6-H); 9.50 (1H, br. s, NH); 12.60 (1H, br. s, OH).
4h	74	234-235	2.50-3.33 (8H, m, $COCH_2CH_2$ & $N(CH_3)_2$); 3.96 (2H, t, CH_2CH_2Cl); 7.03 (1H, d, J = 8.50 Hz, C3-H); 7.46-7.86 (2H, m, C4-H & C6-H); 9.83 (1H, br. s, NH); 10.16 (1H, br. s, OH)

*Protons of COOH, CONH and OH groups are exchangeable by D_2O.

The IR spectra (KBr, cm $^{-1}$) showed stretching bands at:

4a: 3445 (OH), 3270-2500 (broad band of carboxylic OH), 1690 (C=O) and 1665 (C=O; amide)

4b-d: 3410 (OH), 3230 (NH), 1680 (C=O; ester) and 1655 (C=O; amide)

4e-h: 3415 (OH), 3230 (NH) and 1647 (C=O; amide).

Table III. Physical and spectral data of 5-(β-chloropropionylamino) salicylic acid esters and amides (**5a-h**)

Compounds	Yield %	m.p. °C	^{1}H-NMR (DMSO-d_6, δ ppm)*
5a	75	237-239	1.85 (3H, d, $CHCH_3$); 4.83 (1H, q, $CHCH_3$); 7.16 (1H, d, J = 8.50 Hz, C3-H); 7.96 (1H, dd, J = 8.50 Hz, C4-H); 8.43 (1H, d, C6-H); 10.50-11.66 (3H, br. s, NH, OH & COOH)
5b	73	119-120	1.80 (3H, d, $CHCH_3$); 3.95 (3H, s, OCH_3); 4.59 (1H, q, $CHCH_3$); 6.90 (1H, d, J = 8.50 Hz, C3-H); 7.50 (1H, dd, J = 8.50 Hz, C4-H); 8.00 (1H, d, C6-H); 8.45 (1H, br. s, NH); 10.79 (1H, s, OH).
5c	58	110-111	1.43 (3H, t, CH_2CH_3); 1.85 (3H, d, $CHCH_3$); 4.23-4.76 (3H, m, OCH_2CH_3 & $CHCH_3$); 7.00 (1H, d, J = 8.50 Hz, C3-H); 7.63 (1H, dd, J = 8.50 Hz , C4-H); 8.03 (1H, d, C6-H); 8.35 (1H, br. s, NH); 10.85 (1H, s, OH).
5d	53	98-100	0.95 (3H, t, $CH_2CH_2CH_3$); 1.40-2.06 (5H, m, $CH_2CH_2CH_3$ & $CHCH_3$); 4.10-4.70 (3H, m, $OCH_2CH_2CH_3$ & $CHCH_3$); 6.90 (1H, d, J = 8.50 Hz, C3-H); 7.53 (1H, dd, J = 8.50 Hz, C4-H); 7.95 (1H, d, C6-H); 8.23 (1H, br. s, NH); 10.73 (1H, s, OH).
5e	57	210-211	1.75 (3H, d, $CHCH_3$); 4.60 (1H, q, $CHCH_3$); 6.90 (1H, d, J = 8.50 Hz, C3-H); 7.10-7.40 (3H, br. s, $CONH_2$ & C4-H); 7.60 (1H, d, C6-H); 9.45 (1H, br. s, NH); 12.20 (1H, s, OH).
5f	64	206-208	1.73 (3H, d, $CHCH_3$); 2.90 (3H, d, $NHCH_3$); 4.56 (1H, q, $CHCH_3$); 6.86 (1H, d, J = 8.50 Hz, C3-H); 7.49 (1H, dd, J = 8.50 Hz, C4-H); 8.00 (1H, d, C6-H); 8.15 (1H, br. s, $NHCH_3$); 9.55 (1H, br. s, NH); 12.30 (1H, s, OH).
5g	56	170-172	1.25 (3H, t, $NHCH_2CH_3$); 1.80 (3H, d, $CHCH_3$); 3.10-3.85 (2H, m, $NHCH_2CH_3$); 4.75 (1H, q, $CHCH_3$); 7.10 (1H, d, J = 8.50 Hz, C3-H); 7.70 (1H, dd, J = 8.50 Hz, C4-H); 8.06-8.45 (2H, m, C6-H & $NHCH_2CH_3$); 9.90 (1H, br. s, NH); 10.45 (1H, s, OH).
5h	65	224-226	1.66 (3H, d, $CHCH_3$); 3.03 (6H, s, $N(CH_3)_2$); 4.76 (1H, q, $CHCH_3$); 7.03 (1H, d, J = 8.50 Hz, C3-H); 7.46-7.76 (2H, m, C4-H & C6-H); 9.90 (1H, s, NH); 10.40 (1H, s, OH).

*Protons of COOH, CONH and OH groups are exchangeable by D_2O.

The IR spectra (KBr, cm $^{-1}$) showed stretching bands at:

5a: 3450 (OH), 3220-2500 (broad band of carboxylic OH), 1700 (C=O) and 1659 (C=O; amide)

5b-d: 3430 (OH), 3285 (NH) and 1695 (C=O; ester)

5e-h: 3470 (OH), 3205 (NH) and 1659 (C=O; amide).

Table IV. Physical and microanalytical data of 3-carbamoyl-1-(β-*N*-(4'-hydroxy-3'-substituted phenyl)carbamoyl)ethylpyridinium halides (**6a-h**)

Compounds	Yield %	m.p.* °C	Mol. Formula (M. Wt.)	Microanalysis Calculated / Found		
				C%	H%	N%
6a	50	250-252	$C_{16}H_{16}BrN_3O_5$ (410.22)	46.84 46.84	3.93 4.16	10.24 10.26
6b	75	203-204	$C_{17}H_{18}BrN_3O_5$ (424.24)	48.12 48.20	4.27 4.55	9.90 10.05
6c	73	199-200	$C_{18}H_{20}BrN_3O_5$ (438.27)	49.32 49.62	4.60 4.77	9.59 9.73
6d	76	210-211	$C_{19}H_{22}BrN_3O_5$ (452.30)	50.45 50.98	4.90 5.15	9.29 9.41
6e	65	218-220	$C_{16}H_{17}BrN_4O_4$ (409.23)	46.95 46.95	4.18 4.39	13.69 13.66
6f	67	216-218	$C_{17}H_{19}BrN_4O_4$ (423.26)	48.24 48.07	4.52 4.81	13.23 13.07
6g	72	174-176	$C_{18}H_{21}BrN_4O_4$ (437.28)	49.44 49.63	4.84 5.13	12.81 12.95
6h	77	222-224	$C_{18}H_{21}ClN_4O_4.1/2\ H_2O$ (401.84)	53.80 53.45	5.51 5.64	13.94 13.65

*__6a__, __e__, __f__, and __g__ were crystallized from mixture of methanol/chloroform (3:1) and the rest of compounds from a mixture of methanol/ether (2:1).

acacia. The reaction time was evaluated directly before and after 1/2, 1, 2, 3, and 5 h of compounds injection.

Results of analgesic activity of the test compounds and 5-ASA are listed in Table IX.

Gastric ulcerogenic effects (Duffy *et al.*, 2001; Singh *et al.*, 1984)

Male adults albino rats (120-200 g), were divided into groups each of five animals. Animals were starved but had free access to water for 24 h before the experiment. Animals were then treated orally by means of a stomach tube with solutions or suspensions of the test compound and aspirin as a reference drug in 5% aqueous solution of gum acacia at a dose level of 100 mg/kg. Control animals were treated with an equal volume of 5% gum acacia. After 6 h, the rats were sacrificed and the stomach was removed, dissected along the greater curvature and washed in tap water. The lesions on gastric mucosa, if any, were counted by visual examination under magnification. Gastric ulcerogenicity of **7f** was compared with that of aspirin (Table X).

Determination of acute toxicity (LD_{50}) (Sztaricskai *et al.*, 1999)

Groups of male adult albino mice, each of five animals (25-30 g), were injected i.p. with graded doses of **7f**. The percentage of mortality, in each group of animals, was determined, 72 h later to injection. Computation of LD_{50} was processed by a graphical method.

Hydrolytic cleavage of 5-ASA prodrugs by intestinal microflora

***In vitro* study** (Jung *et al.*, 2000)

Incubation of **7f**, sulfasalazine, or 5-ASA with the cecal and colonic contents of rats

The cecal and colonic segments of the intestines of male Sprague-Dawely rats weighing between (120-150 g) were cut open and their contents were distributed rapidly into microtubes (0.2 g each). 0.45 mL of the solutions of the test compounds in an isotonic phosphate buffer pH 6.8 corresponding to 65 mg of 5-ASA was added. The mixture was incubated at 37°C in an anaerobic jar for 24 h. Incubation mixtures from which either the cecal contents or the compounds were omitted served as controls. The microtubes were taken out and centrifuged at 8000 r.p.m. to obtain clear supernatants. The released 5-ASA and intact compound in each incubation mixture were detected by TLC using *n*-butanol: acetic acid: water (4:1:1) as a mobile phase in comparison to authentic samples of the test compounds. TLC plates were visualized by UV at 254 nm.

Bacteriological testing (Peppercorn *et al.*, 1972)

Streptococcus pyogenus cultivated on blood agar medium was further cultivated in cooked meat medium (Atlas *et al.*, 1997) (1/2 mg in 5 mL brain heart infusion) by incubation for 48 h. The compound (0.5 mg/mL) in phosphate buffer pH 6.8 was added to the suspension of the organism (8 mL) and then incubated for one week. Incubation mixtures from which either the bacterial inoculums, or the test compounds were omitted served as controls. After seven days, the test samples were clarified by centrifugation at 8000 r.p.m. and then examined for the released 5-ASA and the intact prodrug, if any, by TLC as previously described.

***In vivo* study** (Jung *et al.*, 2000; Chan *et al.*, 1983)

Release of 5-ASA and *N*-acetyl-5ASA in feces after oral administration of **7f** or sulfasalazine:

Male Sprague-Dawely rats, three for each compound, weighing (120-150 g), were housed in individual metabolic cages and starved for 24 h before experiments but had free access to water. The test compound was dissolved or suspended in 5% aqueous solution of gum acacia and was orally administered by an intragastric tube at dose level equivalent to 50 mg/kg of 5-ASA. The fecal samples were collected daily for two days. Each day sample was diluted with isotonic phosphate buffer (pH 6.8) to 10-fold.

Table V. Spectral data of 3-carbamoyl-1-(β-*N*-(4'-hydroxy-3'-substituted phenyl)carbamoyl)ethylpyridinium halides (**6a-h**)

Compounds	^{1}H-NMR (DMSO-d_6, δ ppm)*
6a	3.35 (2H, t, $COCH_2CH_2$); 5.15 (2H, t, $N^+CH_2CH_2$); 7.06 (1H, d, C5'-H, *J* = 8.50 Hz); 7.80 (1H, dd, *J* = 8.50 Hz , C6'-H); 8.15-8.60 (4H, m, C2'-H, C5-H & NH_2); 8.80 (H, br. s, NH); 9.20 (1H, d, *J* = 8.50 Hz , C4-H); 9.53 (1H, d, C6-H); 9.85 (1H, s, C2-H); 10.45-10.85 (2H, br. s, OH & COOH).
6b	3.25 (2H, t, $COCH_2CH_2$); 3.90 (3H, s, OCH_3); 5.00 (2H, t, $N^+CH_2CH_2$); 6.96 (1H, d, C5'-H, *J* = 8.50 Hz); 7.65 (1H, dd, *J* = 8.50 Hz , C6'-H); 8.05-8.45 (3H, m, C2'-H, C5-H & 1H of NH_2); 8.65 (1H, br. s, $CONH_2$); 9.03 (1H, d, *J* = 8.50 Hz , C4-H); 9.36 (1H, d, *J* = 8.00 Hz , C6-H); 9.70 (1H, s, C2-H); 10.13-10.60 (2H, br. s, NH & OH).
6c	1.45 (3H, t, CH_2CH_3); 3.36 (2H, t, $COCH_2CH_2$); 4.50 (2H, q, CH_2CH_3); 5.13 (2H, t, $N^+CH_2CH_2$); 7.05 (1H, d, *J* = 8.50 Hz , C5'-H); 7.80 (1H, dd, *J* = 8.50 Hz , C6'-H); 8.10-8.55 (3H, m, C2'-H, C5-H & 1 H of $CONH_2$); 8.75 (1H, br. s, $CONH_2$); 9.20 (1H, d, *J* = 8.50 Hz , C4-H); 9.45 (1H, d, *J* = 8.00 Hz , C6-H); 9.75 (1H, s, C2-H); 10.20-10.45 (2H, br. s, NH & OH).
6d	1.00 (3H, t, $CH_2CH_2CH_3$); 1.55-2.15 (2H, m, $CH_2CH_2CH_3$); 3.25 (2H, t, $COCH_2CH_2$); 4.30 (2H, q, $OCH_2CH_2CH_3$); 5.10 (2H, t, $N^+CH_2CH_2$); 7.00 (1H, d, *J* = 8.50 Hz , C5'-H); 7.75 (1H, dd, C6'-H); 8.03-8.50 (3H, m, C2'-H, C5-H & 1 H of NH_2); 8.75 (1H, br. s, $CONH_2$); 9.20 (1H, d, *J* = 8.50 Hz, C4-H); 9.40 (1H, d, *J* = 8.00 Hz, C6-H); 9.75 (1H, s, C2-H); 10.25-10.50 (2H, br. s, NH & OH).
6e	3.29 (2H, t, $COCH_2CH_2$); 5.10 (2H, t, $N^+CH_2CH_2$); 6.86 (1H, d, *J* = 8.50 Hz, C5'-H); 7.50 (1H, dd, *J* = 8.50 Hz, C6'-H); 7.80-8.45 (4H, m, C2'-H, C5-H & $CONH_2$); 8.65 (2H, br. s, $CONH_2$); 9.03 (1H, d, *J* = 8.50 Hz, C4-H); 9.36 (1H, d, *J* = 8.00 Hz, C6-H); 9.70 (1H, s, C2-H); 10.20-10.40 (2H, br .s, NH & OH).
6f	2.96 (3H, d, $NHCH_3$); 3.35 (2H, t, $COCH_2CH_2$); 5.20 (2H, t, $N^+CH_2CH_2$); 7.03 (1H, d, *J* = 8.50 Hz, C5'-H); 7.65 (1H, dd, *J* = 8.50 Hz, C6'-H); 8.16 (1H, d, C2'-H); 8.25-8.65 (2H, m, C5-H & $NHCH_3$); 8.85 (2H, br. s, $CONH_2$); 9.23 (1H, d, *J* = 8.50 Hz, C4-H); 9.60 (1H, d, C6-H, *J* = 8.00 Hz); 10.20 (1H, s, C2-H); 10.60 (1H, br. s, NH); 12.35 (1H, br. s, OH).
6g	1.15 (3H, t, $NHCH_2CH_3$); 3.05-3.70 (4H, m, $NHCH_2CH_3$ & $COCH_2$ CH_2); 5.15 (2H, t, $N^+CH_2CH_2$); 7.05 (1H, d, *J* = 8.50 Hz, C5'- H); 7.66 (1H, dd, *J* = 8.50 Hz, C6'-H); 8.15 (1H, d, C2'-H); 8.20-8.60 (2H, m, C5-H and $NHCH_2CH_3$); 8.70-9.00 (2H, br. s, $CONH_2$); 9.25 (1H, d, *J* = 8.50 Hz, C4-H); 9.56 (1H, d, *J* = 8.00 Hz, C6-H); 9.90 (1H, s, C2-H); 10.33-12.00 (2H, br. s, NH & OH).
6h	3.06 (6H, s, $N(CH_3)_2$); 3.26 (2H, t, $COCH_2CH_2$); 5.21 (2H, t, $N^+CH_2CH_2$); 7.09 (1H, d, *J* = 8.50 Hz, C5'- H); 7.46-7.79 (2H, m, C6'-H & C2'-H); 8.23-8.70 (2H, m, C5-H & 1 H of NH_2); 9.06 (1H, br. s, $CONH_2$); 9.33 (1H, d, *J* = 8.50 Hz, C4-H); 9.63 (1H, d, *J* = 8.00 Hz, C6-H); 10.06 (1H, s, C2-H); 10.30-10.9 (2H, br. s, NH & OH).

*Protons of COOH, CONH and OH groups are exchangeable by D_2O.

The IR spectra (KBr, cm^{-1}) showed stretching bands at:

6a: 3400 (OH), 2280-2500 (carboxylic OH) and 1680 (C=O)

6b-d: 3465 (OH), 3250 (NH), 1690 (C=O; ester) and 1660 (C=O; amide)

6e-h: 3390 (OH), 3200 (NH) and 1670 (C=O; amide).

Mass spectra; m/z (%):

6b: 26.97 (19.9), 54.9 (68.8), 77.86 (27.86), 105.87 (35.5), 134.74 100), 188.6 (78.4), 206.1 (54), and 220.6 (51); FAB: 330.72 (M. Wt. – Br)

6c: 26.91 (17.4), 50.82 (21.6), 54.8 (54.7), 77.72 (42.4), 105.6 (41.3), 133.5 (26.8), 134.9 (97.9), 188.29 (100), and 234.18 (80.9)

The mixture was vortexed and centrifuged at 8000 r.p.m. Aliquots of the clear supernatants were detected by TLC as mentioned above.

pH stability of 7f (Jung *et al.*, 2000)

Chemical stability of **7f** was determined by preparing solutions of **7f** (140 mg/mL) in phosphate buffer pH 6.8 and in hydrochloric acid buffer pH 1.2. The solutions were incubated at 37°C for 6 h. Aliquots of the solutions were taken at one-hour time intervals and examined by TLC for the released 5-ASA and the intact compound as previously described.

RESULTS AND DISCUSSION

Chemistry

The designed compounds are aimed primarily as safe antiinflammatory and analgesic agents and may act as prodrugs for colon specific delivery of 5-ASA due to their ionic nature and several polar groups present which impart hydrophilic characters to the designed prodrugs and inturn minimize their systemic absorption. In addition, the amide bond was reported to be stable in the upper intestine, and thus a large quantity of the orally administered prodrug might be delivered to the colon in an intact form, where it degrades by amidases of microflora to release 5-ASA.

The final compounds (**6a-h** and **7b-h**) were obtained by quaternization of nicotinamide by 5-(haloacylamino) salicylic acid, its esters and its amides (**4a-h** and **5a-h**) in acetonitrile (Scheme 1).

Synthesis of the intermediates (**4a-h** and **5a-h**) was carried out by haloacylation of (**3a-h**). 5-Aminosalicylates (**3b-d**) were prepared by esterification of 5-ASA, while 5-

Table VI. Physical and microanalytical data of 3-carbamoyl-1-(α-*N*-(4'-hydroxy-3'-substituted phenyl)carbamoyl)ethylpyridinium chlorides (**7b-h**)

Compounds	Yield %	m.p.* °C	Mol. Formula (M. Wt.)	Microanalysis Calculated / Found		
				C%	H%	N%
7b	60	169-170	$C_{17}H_{18}ClN_3O_5 . H_2O$ (397.81)	51.32 51.46	5.06 5.47	10.56 10.75
7c	57	244-245	$C_{18}H_{20}ClN_3O_5$ (393.82)	54.89 54.51	5.11 5.38	10.66 10.65
7d	55	248-250	$C_{19}H_{22}ClN_3O_5$ (407.85)	55.95 55.51	5.43 5.74	10.30 10.25
7e	54	158-160	$C_{16}H_{17}ClN_4O_4 . 2 H_2O$ (400.80)	47.95 48.05	5.28 5.30	13.97 13.73
7f	60	180-182	$C_{17}H_{19}ClN_4O_4 . H_2O$ (396.82)	51.45 51.46	5.33 5.86	14.11 14.19
7g	58	206-208	$C_{18}H_{21}ClN_4O_4 . H_2O$ (410.85)	52.62 53.02	5.64 6.06	13.63 13.88
7h	50	184-186	$C_{18}H_{21}ClN_4O_4 . 1.5 H_2O$ (419.85)	51.49 51.37	5.64 5.91	13.34 13.02

*Compounds **7e** and **7f** were crystallized from a mixture of methanol/chloroform (3:1) and the other compounds – from methanol/ether (2:1).

aminosalicylamides (**3e-h**) were prepared by reductive fission of the corresponding azo dyes (**2e-h**) in analogy to reported methods (Conant *et al.*, 1941; Furniss *et al.*, 1989). Structures of the intermediates have been confirmed by IR and NMR spectral data, while those of the final compounds by microanalyses, IR, NMR and some by mass spectrometry as shown in tables (I-VII).

Biological screening

Pharmacological screening

Seven of the synthesized (**6b**, **6f**, **7b**, and **7e-h**) were, preliminarly, screened for:

Anti-inflammatory activity against carrageenin–induced rat paw odema (Winter *et al.*, 1962), results are shown in Table VIII.

Analgesic activity of the same compounds was evaluated by the hot plate method (Eddy *et al.*, 1953), results are listed in Table IX.

Results obtained were statistically analyzed to find the standard errors and student's *t*-test was used to analyze the significance of the obtained results.

Most of the test compounds showed remarkable anti-inflammatory and analgesic activities. Study of the results showed that the *N*-methylamide (**7f**) was more active as anti-inflammatory and analgesic than its constitutional isomer **6f**.

Superiority of both anti-inflammatory and analgesic effects of **7f** indicates that branching of the chain of the spacer ($R^1CH(CH_2)_n$; **6** and **7**, Scheme 1) augments both activities determined. These data are in a good agreement with the fact that the α-CH_3 substituent presents in "profens" increases their cyclooxygenase inhibitory activity and reduces their toxicity than phenacs (Borne, 2002). Moreover, many profens are in use in clinical practice, while very few propionic acid analogues (oxaprozin) are known in the market (Borne, 2002).

The encouraging results obtained for *N*-methylamide (**7f**) of nicotinamide series prompted us to study the anti-inflammatory and analgesic activities of its *N*-alkyl homologues (**7g** and **7h**). Again, the results obtained assured that increase of bulk of alkyl substituent on the amide nitrogen decreases the activity. These results are consistent with similar ones reported in concern of activities of salicylamide and its *N*-alkyl or *N,N*-dialkyl derivatives (Way *et al.*, 1953).

It is clear that **7f** has the most promising anti-inflammatory and analgesic activities in both series of testing, so it was subjected to further pharmacological studies including gastric ulcerogenicity and determination of the median lethal dose (LD_{50}).

Ulcerogenic effects

Gastric ulcerogenic effect was determined in rats, for the most active analgesic and anti-inflammatory compound **7f** and results are recorded in Table X.

Test **7f** didn't show any signs of gastric lesions, while aspirin treated animals showed high incidence of mucosal lesions, which vary from redness of mucosa, hyperemia, petichiae to pin point ulcers. These results are consistent with the known beneficial effects of 5-ASA and its derivatives on gastrointestinal tract inflammation[5] along with promotion of endogenous cytoprotective prostaglandins (Hoult *et al.*, 1981).

Acute toxicity (LD_{50})

LD_{50} of the investigated **7f** was determined using graphical method. LD_{50} of **7f** was found to be 800 mg/kg (i.p.), while those of 5-ASA is (469 mg/kg i.p.) (Sztaricskai *et al.*, 1999) and aspirin is (533 mg/kg i.p.) (Abdel-Alim *et al.*, 1980).

Hydrolytic Cleavage of 5-ASA Prodrugs by Intestinal Microflora

The role of gut flora in drug metabolism (Abu Shamat, 1993) can be investigated *in vitro* by incubating the drug with gut contents of an animal or man in a suitable medium or by incubating the drug with bacterial strain simulating those found in the large intestine of the human and rodents. The outcome of this direct approach is usually dependent upon successful cultivation of the anaerobic microflora under conditions similar to gut environment with regard to the pH and redox potential. Evidence for metabolism by the intestinal microflora *in*

Table VII. Spectral data of 3-carbamoyl-1-(α-*N*-(4'-hydroxy 3'-substituted phenyl) carbamoyl)ethylpyridinium chlorides (**7b-h**)

Compounds	^{1}H-NMR (DMSO-d_6, δ ppm)*
7b	2.23 (3H, d, $CHC\underline{H}_3$); 3.83 (3H, s, OCH_3); 6.43 (1H, q, $N^+C\underline{H}CH_3$); 7.33 (1H, d, *J* = 8.50 Hz, C5'-H); 8.00 (1H, dd, *J* = 8.50 Hz, C6'-H); 8.23-8.76 (2H, m, C2'-H & C5-H); 9.20 (2H, br. s, $CONH_2$); 9.43 (1H, d, *J* = 8.50 Hz, C4-H); 9.73 (1H, d, *J* = 8.00 Hz, C6-H); 10.00 (1H, s, C2-H); 10.76 (1H, br. s, NH); 11.93 (1H, br. s, OH).
7c	1.35 (3H, t, $OCH_2C\underline{H}_3$); 2.20 (3H, d, $CHC\underline{H}_3$); 4.40 (2H, q, $OC\underline{H}_2CH_3$); 6.15 (1H, q, $N^+C\underline{H}CH_3$); 6.95 (1H, d, *J* = 8.50 Hz, C5'-H); 7.75 (1H, dd, *J* = 8.50 Hz, C6'-H); 8.06-8.46 (2H, m, C5- H & C2'-H); 8.85 (2H, br. s, $CONH_2$); 9.13 (1H, d, *J* = 8.50 Hz, C4-H); 9.40 (1H, d, *J* = 8.00 Hz, C6-H); 9.65 (1H, s, C2-H); 10.40 (1H, br. s, NH); 11.40 (1H, s, OH).
7d	0.95 (3H, t, $CH_2CH_2C\underline{H}_3$); 1.55-2.00 (2H, m, $CH_2C\underline{H}_2CH_3$); 2.10 (3H, d, $CHC\underline{H}_3$); 4.25 (2H, t, $OC\underline{H}_2CH_2CH_3$); 6.10 (1H, q, *J* = 7.00 Hz, $N^+C\underline{H}CH_3$); 6.95 (1H, d, *J* = 8.50 Hz, C5'-H); 7.75 (1H, dd, *J* = 8.50 Hz, C6'-H); 8.03-8.45 (2H, m, C2'-H & C5-H); 8.80 (2H, br. s, $CONH_2$); 9.13 (1H, d, C4-H); 9.40 (1H, d, C6-H); 9.65 (1H, s, C2-H); 10.45 (1H, s, NH); 11.35 (1H, s, OH).
7e	2.15 (3H, d, $CHC\underline{H}_3$); 6.26 (1H, q, $N^+C\underline{H}CH_3$); 7.13 (1H, d, *J* = 8.50 Hz, C5'-H); 7.80 (1H, dd, *J* = 8.50 Hz, C6'-H); 8.00-8.75 (4H, m, C5-H , C2'-H & $CONH_2$); 9.15 (2H, br. s, $CONH_2$); 9.36 (1H, d, C4-H); 9.65 (1H, d, *J* = 6.60 Hz, C6-H); 9.90 (1H, s, C2-H); 11.60 (1H, br. s, NH); 12.75 (1H, br. s, OH).
7f	2.15 (3H, d, $CHC\underline{H}_3$); 2.90 (3H, d, $NHC\underline{H}_3$); 6.15 (1H, q, $N^+C\underline{H}CH_3$); 6.96 (1H, d, *J* = 8.50 Hz, C5'-H); 7.65 (1H, dd, *J* = 8.50 Hz, C6'-H); 8.05-8.83 (3H, m, C2'-H and $N\underline{H}CH_3$ & C5-H); 8.93 (2H, br. s, NH_2); 9.20 (1H, d, *J* = 8.50 Hz, C4-H); 9.43 (1H, d, *J* = 8.00 Hz, C6-H); 9.76 (1H, s, C2-H); 11.30 (1H, s, NH); 12.20 (1H, s, OH).
7g	1.25 (3H, t, $CH_2C\underline{H}_3$); 2.10 (3H, d, $CHC\underline{H}_3$); 3.06-3.75 (2H, m, $CH_2C\underline{H}_2CH_3$); 6.25 (1H, q, $N^+C\underline{H}CH_3$); 7.20 (1H, d, *J* = 8.50 Hz, C5'-H); 7.75 (1H, dd, *J* = 8.50 Hz, C6'-H); 8.16-8.70 (2H, m, C2'-H & $N\underline{H}CH_2CH_3$); 8.80-9.16 (3H, m, C5-H & NH_2); 9.35 (1H, d, *J* = 8.50 Hz, C4-H); 9.65 (1H, d, C6-H); 9.90 (1H, s, C2-H); 11.55 (1H, s, NH); 12.60 (1H, s, OH).
7h	2.10 (3H, d, $CHC\underline{H}_3$); 2.95 (6H, s, $N(CH_3)_2$); 6.20 (1H, q, $N^+C\underline{H}CH_3$); 7.03 (1H, d, J = 8.50 Hz, C5'-H); 7.43-7.80 (2H, m, C6'-H & C2'-H); 8.06-8.66 (1H, m, C5-H); 8.96 (2H, br. s, NH_2); 9.26 (1H, d, *J* = 8.50 Hz, C4-H); 9.60 (1H, d, *J* = 8.00 Hz, C6-H); 9.86 (1H, s, C2-H); 10.15 (1H, br. s, NH); 11.50 (1H, br. s, OH).

*Protons of CONH and OH groups are exchangeable by D_2O.

The IR spectra (KBr, cm $^{-1}$) showed stretching bands at:

7b-d: 3350 (OH), 3260 (NH) and 1670 (C=O)

7e-h: 3390 (OH), 3265 (NH) and 1680 (C=O; amide).

Table VIII. Anti-inflammatory activity of some 3-carbamoyl-1-(*N*-(4'-hydroxy-3'-Substituted phenyl)carbamoyl)alkylpyridinium chlorides against carrageenin-induced rat paw odema

No.	Volume of the right paw (mm) at different times after carrageenin injection (time in hours).					
	0	1/2	1	2	3	5
Control	3.1 ±0.02	5.9 ±0.18	6.3±0.19	6.4 ±0.2	6.9±0.2	7 ±0.16
6b	3.06±0.02	4.8 ±0.3*	4.8±0.18**	4.6** ±0.1	4.5 ±0.2**	5.8±0.2**
7b	3.05±0.01	4.9 ±0.1*	4.8±0.12**	4.8** ±0.1	4.9±0.2**	6.2±0.1
6f	3.01±0.01	4.5 ±0.1**	4.2±0.19**	4.6** ±0.2	5.6± 0.2**	5.4±0.1**
7e	3 ±0.01	5.25±0.5	4.8±0.43**	5.62 ±0.4*	6.1±0.6	6.3±0.6
7f	2.99±0.02	4.1 ±0.1**	3.7±0.1**	4** ±0	4.8± 0.1**	5.1±0.2**
7g	3 ±0.04	4.5 ±0.2**	4.6±0.3**	4.7** ±0.4	5.5 ± 0.5**	6.2±0.3
7h	3.03±0.04	4.7 ±0.2**	5 ±0.16**	5.3 ±0.3*	6.1± 0.2	6.3±0.24
5-ASA	2.98±0.1	4.3 ±0.2**	4.1±0.1**	4.2 ±0.2**	4.4± 0.2**	6.2±0.75
SASP	3.07±0.06	4.7 ±0.1**	4.7±0.1**	4.5 ±0**	4.7±0.2**	5.7±0.3**

- *Values are the mean* ± S. E. *of five observations.*
- * Significant difference at P < 0.05 vs. control value (student's-*t*-test)
- ** Significant difference at P < 0.01 *vs. control value (student's-t-test)*
- The reference drugs, test compounds and gum acacia were injected i.p. into rats (10 mg/kg), 30 minutes before carrageenin injection.

vivo usually relies on the detection of a specific microbial drug metabolite.

In the present work, qualitative determination of the metabolic outcome of the test compounds has been done in rats in comparison to sulfasalazine by *in vivo* and *in vitro* methods. Study involved the ability of release of 5-

Table IX. Analgesic activity of some 3-carbamoyl-1-(*N*-(4'-hydroxy-3'-substituted phenyl)carbamoyl)alkylpyridinium chlorides on heat-induced pain in mice

No.	The average reaction time (second) at different times after compound administration.				
	1/2 h	1 h	2 h	3 h	5 h
control	8.6 ±0.74	8.1 ±0.74	9 ±1	8 ±1.29	7 ±0.16
6b	15.5 ±1**	19.25±1**	22 ±1.47**	40 ±2.7**	22 ±0.9**
7b	10.87±0.6	14.6 ±1**	21.2 ±1.7**	29 ±2.3**	14 ±1.3**
6f	19.5 ±1.8**	19.7 ±1.9**	22.6 ±1.1**	25 ±0.6**	14.7 ±1**
7e	9 ±0.5	11 ±1.1*	13 ±1.2**	14.7±1**	13.5 ±0.7**
7f	19.7 ±1.5**	19 ±0.4**	22 ±1.5**	28 ±0.6**	24 ±1.6**
7g	12 ±0.8**	13 ±0.7**	15.98±1**	21 ±1.4**	22.15±1**
7h	13.8 ±1.3**	16.1 ±1**	18.9 ±1.8**	19 ±1.85**	20.5 ±2**
5-ASA	12.5 ±1.2**	23.1 ±2**	31.9 ±2.5**	45 ±1.4**	9.1 ±0.95

- Values are the mean ± S. E. of five observations.
- *Significant difference at $P < 0.05$ vs. control value (student's-*t*-test)
- **Significant difference at $P < 0.01$ vs. control value (student's *t*-test)
- The reference drug, test compounds and gum acacia were injected intraperitoneally into rats (10 mg/kg) 30 minutes before testing.

ASA as a hydrolytic product of **7f** under the effect of microflora.

***In vitro* study**

Release of 5-ASA after incubation of **7f** with cecal and colonic contents of rats: When **7f** was incubated with the cecal contents, 5-ASA was released similarly to that released from sulfasalazine and was detected by TLC (Table XI). This indicates that the microflora are able to release 5-ASA from the designed prodrugs and activation can take place most readily in the rat cecum. *N*-Acetyl-5-ASA couldn't be detected in this test.

Bacteriological study:

It has been reported that the predominant intestinal microflora are those of the non-sporing, strictly, anaerobes (Abu Shamat, 1993).

In this work, analysis of incubation mixture (**7f** with Streptococcus pyogenus in cooked meat medium) showed considerable disappearance of the test prodrug together with the appearance of the released 5-ASA as indicated by TLC (Table XI). This finding indicates that the amide linkages of the test compounds are able to be hydrolyzed by the intestinal microflora (Abu Shamat, 1993).

Table X. Ulcerogenic effects of **7f** in comparison to aspirin

	No. of animals with the lesions	Total No. of lesions
7f	0	0
Aspirin	4	4
Vehicle	0	0

Table XI. R_f values of the test compound and reference standards

	5-ASA	N-acetyl-5-ASA	7f	SASP
R_f	0.63	0.80	0.31	0.85

***In vivo* study**

Recovery of 5-ASA and *N*-acetyl-5-ASA in the feces after oral administration of **7f** or sulfasalazine along with the intact compounds has been detected by TLC. 5-ASA and *N*-acetyl-5-ASA were recovered in feces, after hydrolysis of the given prodrug **7f** in addition to trace amounts of intact **7f** as indicated by their R_f values after visualization by UV at 254 nm as shown in Table XI.

pH stabiltiy of 7f

pH stability of **7f** was determined to confirm that the release of 5-ASA is due to bacterial metabolism not due to chemical hydrolysis in the stomach (pH 1.2) or in the small intestine (pH 6.8). No 5-ASA was detected during the 6 h of the incubation period with both buffers. This indicates that **7f** might be chemically stable during the transit through the gastrointestinal tract to the colon, similar results have been, previously, reported for salicylamide and its derivatives during transit through GIT (Way, 1953).

In view of the results of pharmacological testing, *in vitro*, *in vivo* hydrolysis and pH stability of the prepared prodrugs, it is worthy to mention that release and delivery of 5-ASA (the target drug) to the colon has been realized in addition to the anti-inflammatory and analgesic activities of these compounds.

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