Bull. Pharm. Sci., Assiut University, Vol. 28, Part 2, December 2005, pp. 237-253.

SYNTHESIS OF TRIGONELLINE AND NICOTINAMIDE LINKED PRODRUGS OF 5-AMINOSALICYLIC ACID (5-ASA) WITH ANALGESIC AND ANTI-INFLAMMATORY EFFECTS

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يتناول هذا البحث تخليق يوديدات ٣ ن(4` هيدروكسى ٣` مستبدل فينيل) كارباموبل ١ ميثيل البيردينيم (مركبات **5b-j**) وكلوريدات ٣ كارباموبل (١ ن(4` هيدروكسى ٣` مستبدل فينيل) كارباموبل)- ميثيل البيردينيم (مركبات **7a-j**). ولقد تم التأكد من التركيب البنائى للمركبات الوسيطة بالأشعة تحت الحمراء وأشعة الرنين النووي المغناطيسي أما المركبات النهائية فقد تم التأكد من تركيبها بواسطة التحليل الدقي للعناصر والأشعة تحت الحمراء ، أشعة الرنين النووي المغناطيسي وبعض منها بأشعة مطياف الكتلة . تم دراسة الفاعلية الفارماكولوجية المبدئيةلتلك المركبات كمضادات للالتهابات ومسكنات للآلام. وأظهر المركب رقم (**5b**) أفضل النتائج ولذلك فقد تم تقييم تأثيره التقرحي علي المعدة وكذلك سميته الحادة وقد ثبت أن هذا المركب آمن وليس له أي تأثير تقرحي علي المعدة مقارنة بعقارى السلفاسالازين وحامض ٥ أمينو ساليسليك. كذلك تم دراسة التحلل المائي للمركب (**5b**) في المعمل وفي حيوانات التجارب وأسفرت الدراسة عن انطلاق حامض الـ٥ أمينو ساليسليك.

*3-N-(4`-Hydroxy-3`-substituted phenyl)carbamoyl-1-methylpyridinium iodides (compds. **5b-j**) and 3-carbamoyl-1-(N-(4`-hydroxy- 3`-substituted phenyl)carbamoyl) methyl pyridinium chlorides (compds. **7a-j**) were synthesised and some of them were tested for their analgesic and antiinflammatory activities by hot plate test and carageenin-induced hind paw edema model, respectively. Compound **5b** revealed the most potent analgesic and anti-inflammatory activities in comparison to sulfasalazine (SASP) and 5-ASA. In addition, ulcerogenicity, LD_{50}, in-vivo and in vitro cleavage and pH stability of compound **5b** were also determined.*

INTRODUCTION

5-Aminosalicylic acid (5-ASA) (mesalamine®)[1] is a substitute for sulfasalazine (SASP)[2,3] in the treatment of inflammatory bowel diseases (IBD) with minor side effects but suffers from some stability problems and rapid absorption from the upper part of the G. I. T.[4] Accordingly several prodrugs, aiming at the delivery of effective therapeutic level of 5-ASA to the colon have been introduced. Coupling of 5-ASA to the carrier molecules was achieved, most commonly, azo, glycosidic, amide or ester linkages. All these linkages are cleaved in the large intestine by the action of microflora.[5] So, prodrugs based on azo-reductase activity of colonic microflora have been developed such as olsalazine (Dipentum®)[6] and balsalazide (Colazide® or Colazal®).[7] The unknown safety and toxicity of some of the substrates for azo reductase activity[8] and the reported antiandrogenic activity of substituted azo benzenes as well as a possible connection between intestinal tumors and the use of the azo dyes are the main drawbacks of the azo prodrugs of 5-ASA.[9] Therefore, the use of the more commonly occurring natural substrates may offer important advantages and hence several prodrugs of 5-ASA have been investigated depending on the high hydrolytic activity of the intestinal microflora such as esters,[10] amides[11] and glycosides.[12]

In a previous work,[13] we reported the synthesis and biological activity of some nicotinamide-linked prodrugs of 5-ASA. These compounds (**Ia-h** and **IIb-h**) are quaternary

Received in 11/4/2005 & Accepted in 17/5/2005
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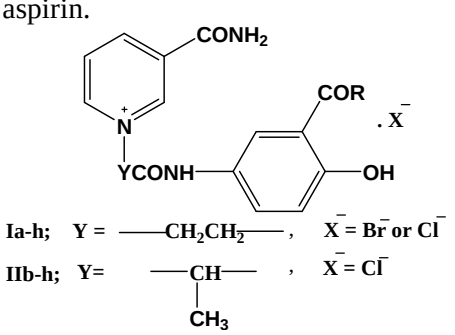
This work has been presented before Assiut Univ. 4th Pharm. Sci. Conf., March 6-7, 2004, Assiut, Egypt, Abstract, p. 95.

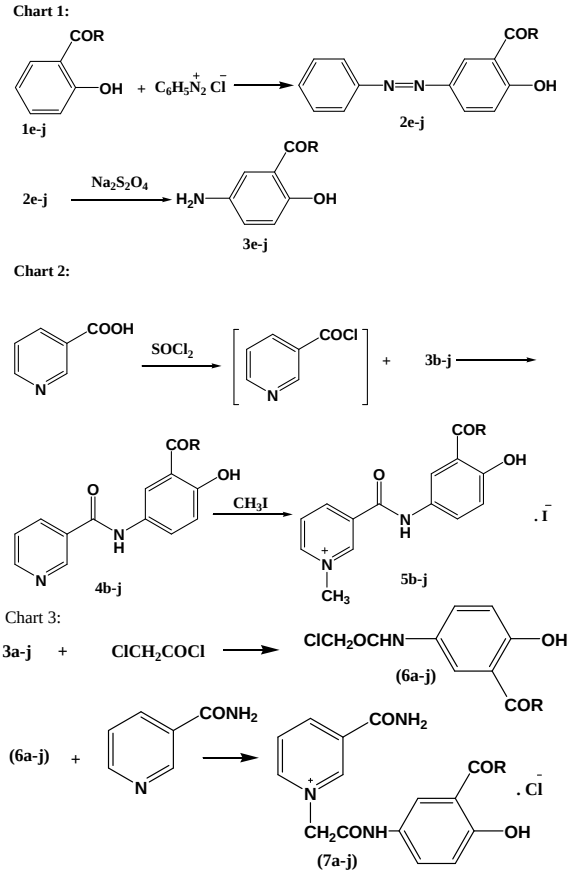
salts incorporating both α- and β-propionamide moieties as spacers between the 5th position of salicylic acid and the endocyclic nitrogen atom of nicotinamide. These compounds exhibited significant and potent analgesic and anti-inflammatory activities with no ulcerogenic effect on the stomach and were twice less toxic than aspirin.



R= OH(**a**), OCH_3(**b**), OC_2H_5(**c**), OC_3H_7(**d**), NH_2(**e**), $NHCH_3$(**f**), NHC_2H_5(**g**), $N(CH_3)_2$(**h**).

In continue, the present work aims at the synthesis of 5-ASA prodrugs of trigonelline (**5b-j**) and nicotinamide (**7a-j**). In the latter compounds, 5-ASA is linked through an acetyl fragment as a spacer to the endocyclic N atom of nicotinamide. The proposed prodrugs embody safe carriers, since 1,4-dihydrotrigonelline and 1,4-dihydronicotinamide were used as chemical delivery system (CDS) to deliver the hydrophilic compounds to the brain and other deep body compartments,[14] in addition both are constituents of vitamin B complex.

The designed compounds (**5** and **7**) are aimed primarily, as safe analgesic and anti-inflammatory agents due to their structural simulation to other reported compounds having the 5-ASA moiety.[15-20] Also, compounds (**5**,**7**) may act as prodrugs for specific delivery of 5-ASA to the colon due to their ionic nature and presence of several polar groups which may impart strong hydrophilic characters to these compounds. In addition, the amide bond was reported[11] to be stable in the upper part of the intestine, and thus a large quantity of the orally administered prodrug might be delivered to the colon, in intact form, where it degrades by amidases of microflora to release 5-ASA.



R= OH(**a**), OCH_3(**b**), OC_2H_5(**c**), OC_3H_7(**d**), NH_2(**e**), $NHCH_3$(**f**), NHC_2H_5(**g**), $N(CH_3)_2$(**h**), piperidino(**i**), morpholino(**j**).

Compounds (**3b-d**) were prepared by direct esterification of **3a**.

Scheme 1

EXPERIMENTAL

Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Precoated silica gel plates 60 F-254 (Merck) were used for TLC monitoring of reactions; spots were detected under UV lamb (MODEL CM-10, U.S.A.).

Infra red 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, and elemental analyses were done on a Perkin-Elmer 240 elemental analyzer at the central laboratory, university of Assiut.

Salicylamides **1e-h**,[21-23] methyl, ethyl, and n-propyl 5-aminosalicylates (**3b-3d**) were prepared according to reported procedures.[24] The physical and spectral data of the n-propyl ester (Table 1) are not found in the available literature, while those of the methyl and ethyl ones are compatible with the reported.[24]

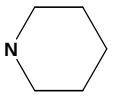
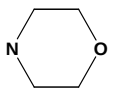
Adult male albino mice and rats were obtained from animal house of the faculty of medicine, University of Assiut.

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

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

Male Sprague-Dawely rats were obtained from the animal house of the National Organization for Bioproducts and Vaccines in Helwan.

Table 1: Physical and spectral data of 5-aminosalicylic acid derivatives (compds. **3d-h**).

Compds. **3**	R	Yield %	M.p°	^{1}H-NMR (DMSO-d_6, δ ppm)*
d	OC_3H_7	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).
e	NH_2	58	186-188	6.63-7.86 (7H; m, 3 aromatic protons, NH_2 & $CONH_2$); 12.55 (1H; s, OH).
f	$NHCH_3$	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).
g	NHC_2H_5	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).
h	$N(CH_3)_2$	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).
i		62	168	1.26-1.86 (6H; br. s, $CH_2CH_2CH_2$ of piperidine); 3.23-4.00 (4H; br. s, CH_2NCH_2 of piperidine); 4.00-4.90 (2H; br. s, NH_2); 6.45 (1H; s, C3-H); 6.70 (2H; s, C4-H & C6-H); 8.80 (1H; br. s, OH).
j		60	188-190	3.20-3.96 (8H; br. s, morpholine); 4.10-5.00 (2H; br. s, NH_2); 6.53 (1H; d, C3-H); 6.75 (2H; dd, C4-H & C6-H); 8.95 (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 i.r. spectra (KBr, cm^{-1}) showed stretching bands at about:

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

Compounds **3e-j** 4485 (OH), 3225 (NH) and 1640 (C=O; amide).

Chemistry

Preparation of 5-aminosalicylamides (compounds 3e-h); general Procedure

a- 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°.

The obtained diazonium salt solution was added slowly with stirring to the cooled alkaline solution of the appropriate salicylamide (compounds **1e-h**; 0.14 mole) in sodium hydroxide (10%, 90 mL). The mixture was further stirred for half an hour below 5°, allowed to stand for three hours at the ambient temperature, and the obtained azo dye (compounds **2e-h**) was filtered, washed with water, dried, and used in the next step without any purification.

b- 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 maintained at 80°, 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 1.

Preparation of 5-(nicotinoyl)aminosalicylic acid esters and amides (compounds 4b-j); general procedure

Thionyl chloride (0.73 mL, 1.18 g, 0.01 mole) was added dropwise to a cooled stirred mixture of nicotinic acid (1.23 g, 0.01 mole) and pyridine (1.62 mL, 0.02 mole) in toluene (10 mL). The reaction mixture was maintained at 90-95° for 1 hr, then cooled, and the appropriate 5-aminosalicylic acid ester or amide (compounds **3b-j**; 0.01 mole) in hot toluene (10 mL) was added portionwise. The reaction mixture was stirred for 4 hours at 90-95°, the toluene layer was decanted, the gummy residue was dissolved in hot water (20 mL), and neutralized with saturated sodium bicarbonate solution. The precipitated product was filtered and crystallized from ethanol (50%). Physical as well as spectral data are shown in Table 2.

Preparation of 3-N-(4`-hydroxy-3`-substituted phenyl)carbamoyl-1-methylpyridinium iodides (compounds 5b-j); general procedure

To a solution of the appropriate 5- (3-pyridylcarbonyl) aminosalicylic acid ester or amide (compounds **4b-j**; 0.002 mole) in acetonitrile (40 mL) methyl iodide (0.3 mL, 0.004 mole) was added. The mixture was refluxed for 7-10 hours, then cooled, the precipitate was filtered, washed with acetonitrile and crystallized from the suitable solvent. Physical, microanalytical, and spectral data are reported in Tables 3 and 4.

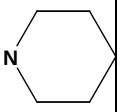
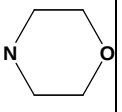
Preparation of 5-(haloacylamino) salicylic acid esters and amides (compounds 6a-j); general procedure

Chloroacetyl chloride (0.02 mole) in dry benzene (5 mL) was added dropwise to a cooled suspension of 5-aminosalicylic acid, its appropriate ester or amide (compounds **3a-j**; 0.02 mole) in dry benzene (50 mL) while stirring. The mixture was refluxed for 24 hours 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 Table 5.

Preparation of 3-carbamoyl-1-(N-(4`-hydroxy-3`-substituted phenyl)carbamoyl) methylpyridinium chlorides (compounds 7a-j); general procedure

Nicotinamide (0.002 mole) and the appropriate chloroacetyl-5-aminosalicylic acid, ester or amide (compounds: **6a-j**; 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) as a developing system. The precipitate was filtered, washed with acetonitrile, and crystallized from the suitable solvent. Physical and microanalytical data are presented in Tables 6 and 7.

Table 2: Physical and spectral data of compounds (**4b-j**).

Compds. **4**	R	Yield %	M.p°	^{1}H-NMR (DMSO-d_6, δ ppm)*
b	OCH_3	80	173	3.96 (3H; s, OCH_3); 6.83 (1H; d, C3-H, J = 8.50 Hz); 7.56-8.00 (2H; m, C4-H & C6-H); 8.26-8.55 (1H; m, C4`-H & C5`-H); 8.90 (1H; d, C6`-H); 9.40 (1H; s, C2`-H); 10.36 (2H; br. s, NH & OH).
c	OC_2H_5	65	123-124	1.35 (3H; t, $CH_2C\underline{H}_3$); 4.36 (2H; q, $OC\underline{H}_2CH_3$); 6.90 (1H; d, C3-H, J = 8.50 Hz); 7.10-7.73 (2H; m, C4-H & C6-H); 8.00-8.40 (2H; m, C4`-H & C5`-H); 8.53-9.00 (2H; m, C6`-H & NH); 9.25 (1H; s, C2`-H); 10.60 (2H; br. s, OH).
d	OC_3H_7	67	131-132	0.95 (3H; t, $CH_2CH_2C\underline{H}_3$); 1.45-2.00 (2H; m, $CH_2C\underline{H}_2CH_3$); 4.30 (2H; t, $OC\underline{H}_2CH_2CH_3$); 6.90 (1H; d, C3-H, J = 8.50 Hz); 7.20-7.70 (2H; m, C4-H & C6-H); 8.00-8.30 (2H; m, C4`-H & C5`-H); 8.40-8.70 (2H; m, C6`-H & NH); 9.06 (1H; s, C2`-H); 10.60 (H; s, OH).
e	NH_2	60	259-260	6.90 (1H; d, C3-H, J = 8.50 Hz); 7.50-8.00 (4H; m, C4-H, C6-H & $CONH_2$); 8.30-8.76 (3H; m, C4`-H, C5`-H & NH); 9.00 (1H; d, C6`-H); 9.45 (1H; s, C2`-H); 10.50 (H; br. s, OH).
f	$NHCH_3$	62	244-246	2.95 (3H; d, $NHC\underline{H}_3$); 7.15 (1H; d, C3-H, J = 8.50 Hz); 7.56-8.13 (2H; m, C4-H & C6-H); 8.33-8.80 (3H; m, C4`-H, C5`-H & $N\underline{H}CH_3$); 8.85-9.16 (2H; m, C6`-H & NH); 9.40 (1H; s, C2`-H); 10.80 (H; br. s, OH)
g	NHC_2H_5	70	186-188	1.35 (3H; t, $CH_2C\underline{H}_3$); 3.25-3.80 (2H; m, $NHC\underline{H}_2CH_3$); 7.20 (1H; d, C3-H, J = 8.50 Hz); 7.66-8.06 (2H; m, C4-H & C6-H); 8.40-8.79 (2H; m, C5`-H & $N\underline{H}CH_2CH_3$); 8.90-9.26 (2H; m, C4`-H & C6`-H); 9.46 (1H; s, C2`-H); 10.76 (1H; br. s, NH); 12.70 (1H; s, OH).
h	$N(CH_3)_2$	75	204-206	3.06 (6H; s, $N(CH_3)_2$); 7.10 (1H; d, C3-H, J = 8.50 Hz); 7.66-8.06 (3H; m, C4-H, C6-H & C5`-H); 8.56 (1H; d, C4`-H, J = 8.50 Hz); 9.00 (1H; d, C6`-H, J = 8.00 Hz), 9.40 (1H; s, C2`-H); 9.70-10.30 (1H; br.s, NH); 10.66 (H; br. s, OH).
i		70	183-185	1.29-1.90 (6H; m, $CH_2CH_2CH_2$ of piperidine); 3.20-4.00 (4H; m, CH_2NCH_2 of piperidine); 7.05 (1H; d, C3-H, J = 8.50 Hz); 7.50-8.00 (3H; m, C4-H, C6-H & C5`-H); 8.50 (1H; d, C4`-H); 8.96 (1H; d, C6`-H); 9.36 (1H; s, C2`-H); 9.90 (1H; s, NH); 10.66 (1H; s, OH).
j		67	222-224	2.95-4.30 (8H; br. s, of morpholine); 7.19 (1H; d, C3-H, J = 8.50 Hz); 7.65-8.13 (3H; m, C4-H, C6-H & C5`-H); 8.60 (1H; d, C4`-H, J = 8.50 Hz); 9.06 (1H; d, C6`-H, J = 8.00 Hz); 9.46 (1H; s, C2`-H); 9.70-10.43 (1H; br. s, NH); 10.75 (1H; s, OH).

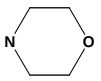
*Protons of CONH and OH groups are exchangeable by D_2O.

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

Compounds **4b-d**: 3420 (OH), 3270 (NH), 1690 (C=O; ester) and 1660 (C=O; amide).

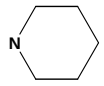
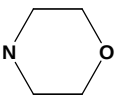
Compounds **4e-j**: 3375 (OH), 3260 (NH) and 1650 (C=O; amide).

Table 3: Physical and microanalytical data of compounds (**5b-j**).

Compds. **5**	R	Yield %	M.p*°	Mol. Formula (M. Wt.)	Microanalysis Calculated / Found		
					C%	H%	N%
b	OCH_3	90	277-279	$C_{15}H_{15}IN_2O_4$ (414.19)	43.49 43.29	3.64 3.63	6.76 6.77
c	OC_2H_5	83	180-182	$C_{16}H_{17}IN_2O_4.1/2\ H_2O$ (437.21)	43.95 43.77	4.14 3.98	6.40 6.36
d	OC_3H_7	80	116-118	$C_{17}H_{19}IN_2O_4.\ H_2O$ (460.30)	44.35 44.49	4.59 4.71	6.08 6.16
e	NH_2	78	130-132	$C_{14}H_{14}IN_3O_3.\ H_2O$ (417.19)	40.30 39.87	3.86 3.97	10.07 10.03
f	$NHCH_3$	85	212-214	$C_{15}H_{16}IN_3O_3$ (413.21)	43.60 43.05	3.90 3.82	10.16 9.99
g	NHC_2H_5	77	206-208	$C_{16}H_{18}IN_3O_3$ (427.24)	44.98 44.88	4.24 4.32	9.83 9.82
h	$N(CH_3)_2$	85	179-181	$C_{16}H_{18}IN_3O_3$ (427.24)	44.98 45.04	4.24 4.59	9.83 9.70
i		87	222-224	$C_{19}H_{22}IN_3O_3.1/2\ H_2O$ (476.28)	47.91 47.83	4.86 5.36	8.82 8.78
j		84	110-112	$C_{18}H_{20}IN_3O_4$ (469.27)	46.07 46.00	4.29 4.96	8.95 8.60

*Compounds **5b-h** were crystallized from a mixture of methanol and chloroform (3:1), **5i-** from acetone and **5j-** from methanol.

Table 4: Spectral data of compounds (**5b-j**).

Compds. **5**	R	^{1}H-NMR (DMSO-d_6, δ ppm)*
b	OCH_3	4.19 (3H; s, OCH_3); 4.79 (3H; s, N^+CH_3); 7.10 (1H; d, C5`-H, J = 8.50 Hz); 8.29 (1H; dd, C6`-H, J = 8.50 Hz); 8.53-8.86 (2H; m, C2`-H & C5-H); 9.26-9.73 (2H; m, C4-H & C6-H); 9.86 (1H; s, C2-H); 10.80 (1H; s, NH); 11.16 (1H; s, OH).
c	OC_2H_5	1.36 (3H; t, $CH_2C\underline{H}_3$); 4.16-4.70 (5H; m, N^+CH_3 & $OC\underline{H}_2CH_3$); 7.03 (1H; d, C5`-H, J = 8.50 Hz); 7.93 (1H; dd, C6`-H, J = 8.50 Hz); 8.13-8.46 (2H; m, C2`-H & C5-H); 8.70-9.26 (2H; m, C4-H & C6-H); 9.55 (1H; s, C2-H); 10.45 (1H; s, NH); 10.70 (1H; s, OH).
d	OC_3H_7	1.00 (3H; t, $CH_2CH_2C\underline{H}_3$); 1.15-2.03 (2H; m, $CH_2C\underline{H}_2CH_3$); 4.15-4.70 (5H; m, $OC\underline{H}_2CH_2CH_3$ & N^+CH_3); 7.00 (1H; d, C5`-H, J = 8.50 Hz); 7.95 (1H; dd, C6`-H, J = 8.50 Hz); 8.23-8.50 (2H; m, C2`-H & C5-H); 8.93-9.30 (2H; m, C4-H & C6-H); 9.56 (1H; s, C2-H); 10.60 (1H; s, NH); 10.90 (1H; s, OH).
e	NH_2	4.66 (3H; s, N^+CH_3); 7.20 (1H; d, C5`-H, J = 8.50 Hz); 7.73-8.00 (5H; m, C6`-H, C2`-H, $CONH_2$ & C5-H); 9.13-9.63 (2H; m, C4-H & C6-H); 9.83 (1H; s, C2-H); 11.04 (1H; s, NH); 12.73 (1H; s, OH).
f	$NHCH_3$	2.90 (3H; d, $NHC\underline{H}_3$); 4.60 (3H; s, N^+CH_3); 7.10 (1H; d, C5`-H, J = 8.50 Hz); 7.93 (1H; dd, C6`-H, J = 8.50 Hz); 8.26-8.70 (2H; m, C2`-H & C5-H); 8.90 (1H; q, $N\underline{H}CH_3$); 9.13-9.56 (2H; m, C4-H & C6-H); 9.75 (1H; s, C2-H); 11.10 (1H; br. s, NH); 12.40 (1H; br. s, OH).
g	NHC_2H_5	1.25 (3H; t, $CH_2C\underline{H}_3$); 3.20-3.85 (2H; m, $NHC\underline{H}_2CH_3$); 4.70 (3H; s, N^+CH_3); 7.29 (1H; d, C5`-H, J = 8.50 Hz); 8.00 (1H; dd, C6`-H, J = 8.50 Hz); 8.40-8.80 (2H; m, C2`-H & C5-H); 9.03 (1H; t, $N\underline{H}CH_2CH_3$); 9.25-9.65 (2H; m, C4-H & C6-H); 9.86 (1H; s, C2-H); 11.15 (1H; s, NH); 12.50 (1H; br. s, OH).
h	$N(CH_3)_2$	3.60 (6H; s, $N(CH_3)_2$); 4.63 (3H; s, N^+CH_3); 7.16 (1H; d, C5`-H, J = 8.50 Hz); 7.66-8.03 (2H; m, C6`-H & C2`-H); 8.36-8.75 (1H; m, C5-H); 9.16-9.60 (2H; m, C4-H & C6-H); 9.83 (1H; s, C2-H); 10.15 (1H; s, NH); 11.00 (1H; s, OH).
i		1.33-1.96 (6H; m, $CH_2CH_2CH_2$ of piperidine); 3.15-3.90 (4H; m, CH_2NCH_2 of piperidine); 4.70 (3H; s, N^+CH_3); 7.13 (1H; d, C5`-H, J = 8.50 Hz); 7.70-8.00 (2H; m, C6`-H & C2`-H); 8.39-8.73 (1H; m, C5-H); 9.19-9.59 (2H; m, C4-H & C6-H); 9.80 (1H; s, C2-H); 10.20-10.70 (1H; br. s, NH); 11.10 (1H; s, OH).
j		3.56 (8H; d, morpholine); 4.60 (3H; s, N^+CH_3); 7.10 (1H; d, C5`-H, J = 8.50 Hz); 7.65-8.15 (2H; m, C6`-H & C2`-H); 8.30-8.73 (1H; m, C5-H); 9.03-9.60 (2H; m, C4-H & C6-H); 9.83 (1H; s, C2-H); 10.13 (1H; br. s, NH); 10.93 (1H; br. s, OH).

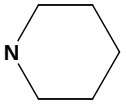
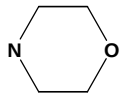
* Protons of CONH and OH groups are exchangeable by D_2O. The I.R. spectra (KBr, cm^{-1}) showed stretching bands at: Compounds **5b-d**: 3440 (OH), 3290 (NH) and 1670 (C=O). Compounds **5e-j**: 33750 (OH), 3260 (NH) and 1648 (C=O; amide). Mass spectra; m/z (%):

Compound **5b**: 50.9 (17.5), 77.9 (54.7), 105.97 (100), 126.6 (31.2), 141.61(78.8), 213.63 (65.9) and 227.6 (26.7).

Compounds **5c**: 50.82(12.5), 77.7 (45), 105.87 (100), 155.28(28.3), 213.19 (20.5), 239 (47.3) and 284.91 (35); FAB: 301.63 (M. Wt. – I) .

Compounds **5d**: 41 (26.7), 43 (43.2), 77.94 (47.9), 105.87 (100), 126.85 (30), 141.85 (49), 239.91 (75.2) and 299.94 (51.3).

Table 5: Physical and spectral data of 5-(chloroacetylamino)salicylic acid esters and amides (compounds **6a-j**).

Compds. **6**	R	Yield %	M.p°	^{1}H-NMR (DMSO-d_6, δ ppm)*
a	OH	77	242-244 as reported [36]	4.25 (2H; s, CH_2); 6.95 (1H; d, C3-H, J = 8.50 Hz); 7.66 (1H; dd, C4-H, J = 8.50 Hz); 8.10 (1H; d, C6-H); 10.20 (1H; br. s, NH); 11.16 (2H; br. s, OH & COOH).
b	OCH_3	80	157	3.95 (3H; s, OCH_3); 4.25 (2H; s, CH_2); 6.95 (1H; d, C3-H, J = 8.50 Hz); 7.65 (1H; dd, C4-H, J = 8.50 Hz); 8.10 (1H; d, C6-H); 10.20 (2H; br. s, NH & OH)
c	OC_2H_5	60	130-131	1.45 (3H; t, $CH_2C\underline{H}_3$); 4.20 (2H; s, CH_2); 4.40 (2H; q, $C\underline{H}_2CH_3$); 6.96 (1H; d, C3-H, J = 8.50 Hz); 7.63 (1H; dd, C4-H, J = 8.50 Hz); 8.00 (1H; d, C6-H); 8.30 (1H; br. s, NH); 10.8 (1H; s, OH).
d	OC_3H_7	68	95-97	1.05 (3H; t, $CH_2CH_2C\underline{H}_3$); 1.50-2.15 (2H; m, $CH_2C\underline{H}_2CH_3$); 4.16 (2H; s, CH_2); 4.30 (2H; t, $OC\underline{H}_2CH_2CH_3$); 7.00 (1H; d, C3-H, J = 8.50 Hz); 7.60 (1H; dd, C4-H, J = 8.50 Hz); 8.00 (1H; d, C6-H); 8.20 (1H; br. s, NH); 10.85 (1H; s, OH).
e	NH_2	62	218-220	4.30 (2H; s, CH_2); 6.90 (1H; d, C3-H, J = 8.50 Hz); 7.53 (1H; dd, C4-H, J = 8.50 Hz); 7.66-8.50 (3H; m, NH_2 & C6-H); 10.15 (1H; br. s, NH); 12.45 (1H; br. s, OH).
f	$NHCH_3$	65	229-231	2.86 (3H; d, $NHC\underline{H}_3$); 4.25 (2H; s, CH_2); 6.86 (1H; d, C3-H, J = 8.50 Hz); 7.5 (1H; dd, C4-H, J = 8.50 Hz); 7.95 (1H; d, C6-H); 8.65 (1H; q, $N\underline{H}CH_3$); 10.25 (1H; br. s, NH); 12.20 (1H; br. s, OH).
g	NHC_2H_5	60	168-170	1.25 (3H; t, $CH_2C\underline{H}_3$); 3.15-3.75 (2H; m, $NHC\underline{H}_2CH_3$); 4.20 (2H; s, CH_2); 7.00 (1H; d, C3-H, J = 8.50 Hz); 7.80 (1H; dd, C4-H, J = 8.50 Hz); 8.20 (1H; d, C6-H); 8.46 (1H; t, $N\underline{H}CH_2CH_3$); 9.85 (1H; br. s, NH); 12.60 (1H; s, OH).
h	$N(CH_3)_2$	65	155-157	3.00 (6H; s, N $(CH_3)_2$); 4.35 (2H; s, CH_2); 7.00 (1H; d, C3-H, J = 8.50 Hz); 7.40-7.70 (2H; m, C4-H & C6-H); 9.86 (1H; br. s, NH); 10.40 (1H; br. s, OH).
i		73	188-190	1.23-2.10 (6H; m, $CH_2CH_2CH_2$ of piperidine); 3.06-3.83 (4H; m, CH_2NCH_2 of piperidine); 4.25 (2H; s, CH_2); 6.95 (1H; d, C3-H, J = 8.50 Hz); 7.36-7.73 (2H; m, C4-H & C6-H); 9.80 (1H; br. s, NH); 10.40 (1H; br. s, OH).
j		75	221-222	3.63 (8H; br. d, morpholine); 4.33 (2H; s, CH_2); 7.06 (1H; d, C3-H, J = 8.50 Hz); 7.50-7.83 (2H; m, C4-H & C6-H); 10.00 (1H; br. s, NH); 10.50 (1H; br. s, OH).

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

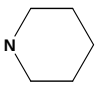
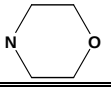
The i.r. spectra (KBr, cm^{-1}) showed stretching bands at:

Compound **6a**: 3425 (OH), 3200-2500 (broad band of carboxylic OH), 1690 (C=O; ester) and 1665 (C=O; amide).

Compounds **6b-d**: 3425 (OH), 3265 (NH), 1700 (C=O; ester) and 1677 (C=O; amide).

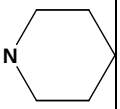
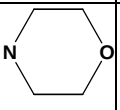
Compounds **6e-j**: 3420 (OH), 3275 (NH) and 1665 (C=O; amide).

Table 6: Physical and microanalytical data of 3-carbamoyl-1-(N-(4`-hydroxy-3`-substituted phenyl) carbamoyl)methylpyridinium chlorides (compounds **7a-j**).

Compds. **7**	R	Yield %	M.p*°	Mol. formula (M. Wt.)	Microanalysis Calculated / Found		
					C%	H%	N%
a	OH	60	246-248	$C_{15}H_{14}ClN_3O_5.1/2\ H_2O$ (360.75)	49.94 50.45	4.19 4.04	11.64 11.85
b	OCH_3	85	233-234	$C_{16}H_{16}ClN_3O_5$ (365.77)	52.54 52.37	4.40 4.61	11.48 11.50
c	OC_2H_5	82	218-220	$C_{17}H_{18}ClN_3O_5.1/2\ H_2O$ (388.79)	52.51 52.75	4.92 5.37	10.80 10.88
d	OC_3H_7	80	235-237	$C_{18}H_{2O}ClN_3O_5$ (393.82)	54.89 54.26	5.11 5.57	10.66 10.63
e	NH_2	77	242-243	$C_{15}H_{15}ClN_4O_4$ (350.76)	51.35 51.19	4.31 4.45	15.97 15.94
f	$NHCH_3$	75	254-255	$C_{16}H_{17}ClN_4O_4.\ H_2O$ (382.79)	50.20 50.76	5.00 5.13	14.63 14.92
g	NHC_2H_5	80	254-256	$C_{17}H_{19}ClN_4O_4.\ H_2O$ (396.82)	51.45 51.37	5.33 5.41	14.11 14.19
h	$N(CH_3)_2$	75	188-190	$C_{17}H_{19}ClN_4O_4.\ 1/2\ H_2O$ (387.81)	52.65 52.80	5.19 5.52	14.44 14.34
i		74	204-206	$C_{20}H_{23}ClN_4O_4.\ H_2O$ (436.88)	54.98 55.51	5.76 5.96	12.82 12.82
j		70	264-265	$C_{19}H_{21}ClN_4O_5$ (420.82)	54.22 54.03	5.02 5.37	13.31 13.26

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

Table 7: Spectral data of compounds (**7a-j**).

Compds. **7**	R	^{1}H-NMR (DMSO-d_6, δ ppm)*
a	OH	6.05 (2H; s, CH_2); 7.10 (1H; d, C5`-H, J = 8.50 Hz); 7.96 (1H; dd, C6`-H, J = 8.50 Hz); 8.25-8.80 (4H; m, C2`-H, C5-H & $CONH_2$); 9.10 (1H; br. s, NH); 9.25-9.65 (2H; m, C4-H & C6-H); 10.15 (1H; s, C2-H); 11.70 (2H; br. s, OH & COOH).
b	OCH_3	3.96 (3H; s, OCH_3); 5.95 (2H; s, CH_2); 7.00 (1H; d, C5`-H, J = 8.50 Hz); 7.76 (1H; dd, C6`-H, J = 8.50 Hz); 8.05-8.50 (2H; m, C2`-H & C5-H); 8.85 (2H; br. s, $CONH_2$); 9.10-9.45 (2H; m, C4-H & C6-H); 9.76 (1H; s, C2-H); 10.40 (1H; s, NH); 11.40 (1H; s, OH).
c	OC_2H_5	1.35 (3H; t, $CH_2C\underline{H}_3$); 4.36 (2H; q, $OC\underline{H}_2CH_3$); 5.86 (2H; s, CH_2); 6.95 (1H; d, C5`-H, J = 8.50 Hz); 7.75 (1H; dd, C6`-H, J = 8.50 Hz); 8.06-8.50 (2H; m, C2`-H & C5-H); 8.85 (2H; br. s, $CONH_2$); 9.06-9.40 (2H; m, C4-H & C6-H); 9.70 (1H; s, C2-H); 10.55 (1H; s, NH); 11.40 (1H; s, OH).
d	OC_3H_7	0.95 (3H; t, $CH_2CH_2C\underline{H}_3$); 1.5-195 (2H; m, $CH_2C\underline{H}_2CH_3$); 4.25 (2H; t, $OC\underline{H}_2CH_2$ CH_3); 5.85 (2H; s, CH_2); 7.00 (1H; d, C5`-H, J = 8.50 Hz); 7.73 (1H; dd, C6`-H, J = 8.50 Hz); 8.00-8.45 (2H; m, C2`-H & C5-H); 8.75 (2H; br. s, $CONH_2$); 8.95-9.30 (2H; m, C4-H & C6-H); 9.60 (1H; s, C2-H); 10.40 (1H; s, NH); 11.30 (1H; s, OH).
e	NH_2	5.85 (2H; s, CH_2); 6.95 (1H; d, C5`-H, J = 8.50 Hz); 7.60 (1H; dd, C6`-H, J = 8.50 Hz); 7.73-8.60 (4H; m, C2`-H, C5-H & NH_2); 8.85 (2H; br. s, NH_2); 9.05-9.35 (2H; m, C4-H & C6-H); 9.70 (1H; s, C2-H); 11.10 (1H; s, NH); 12.35 (1H; s, OH).
f	$NHCH_3$	2.83 (3H; d, $NHC\underline{H}_3$); 5.85 (2H; s, CH_2); 6.95 (1H; d, C5`-H, J = 8.50 Hz); 7.56 (1H; dd, C6`-H, J = 8.50 Hz); 7.93-8.50 (3H; m, C2`-H, C5-H & $N\underline{H}CH_3$); 8.56-9.00 (2H; br. s, $CONH_2$); 9.05-9.40 (2H; m, C4-H & C6-H); 9.70 (1H; s, C2-H); 11.25 (1H; s, NH); 12.25 (1H; s, OH).
g	NHC_2H_5	1.15 (3H; t, $CH_2C\underline{H}_3$); 3.10-3.73 (2H; m, $NHC\underline{H}_2CH_3$); 5.95 (2H; s, CH_2); 7.13 (1H; d, C5`-H, J = 8.50 Hz); 7.76 (H, dd, C6`-H, J = 8.50 Hz); 8.20-8.75 (3H; m, C2`-H, C5-H & $N\underline{H}CH_2CH_3$); 9.00 (2H; br. s, $CONH_2$); 9.20-9.60 (2H; m, C4-H & C6-H); 9.86 (1H; s, C2-H); 11.30 (1H; s, NH); 12.40 (1H; s, OH).
h	$N(CH_3)_2$	2.96 (6H; s, $N(CH_3)_2$); 5.96 (2H; s, CH_2); 7.13 (1H; d, C5`-H, J = 8.50 Hz); 7.53-7.80 (2H; m, C6`-H & C2`-H); 8.26-8.73 (2H; m, C5-H and 1 H of $CONH_2$); 9.05 (1H; br. s, $CONH_2$); 9.30-9.65 (2H; m, C4-H & C6-H); 9.93 (1H; s, C2-H); 10.13 (1H; br. s, NH); 11.43 (1H; br. s, OH).
i		1.50 (6H; m, $CH_2CH_2CH_2$ of piperidine); 3.50 (4H; m, CH_2NCH_2 of piperidine); 5.85 (2H; s, CH_2); 7.03 (1H; d, C5`-H, J = 8.50 Hz); 7.35-7.70 (2H; m, C6`-H & C2`-H); 8.16-8.63 (2H; m, C5-H & 1H of $CONH_2$); 8.95 (1H; br. s, $CONH_2$); 9.20-9.55 (2H; m, C4-H & C6-H); 9.80 (1H; s, C2-H); 9.95 (1H; br. s, NH); 11.50 (1H; s, OH).
j		3.56 (8H; d, morpholine); 5.96 (2H; s, CH_2); 7.10 (1H; d, C5`-H, J = 8.50 Hz); 7.50-7.86 (2H; m, C6`-H & C2`-H); 8.25-8.73 (2H; m, C5`- H & 1H of $CONH_2$); 9.00 (1H; br. s, CO NH_2); 9.26-9.69 (2H; m, C4-H & C6-H); 9.93 (1H; s, C2-H); 10.15 (1H; s, NH); 11.40 (1H; s, OH).

*Protons of COOH, CONH and OH groups are exchangeable by D_2O. The i.r. spectra (KBr, cm^{-1}) showed stretching bands at:

Compound **7a**: 3465 (OH), 3255-2500 (carboxylic OH) and 1662 (C=O).

Compounds **7b-d**: 3420 (OH), 3265 (NH), 1700 (C=O; ester) and 1676 (C=O; amide).

Compounds **7e-j**: 3390 (OH), 265 (NH) and 1681 (C=O; amide). Mass spectra; m/z (%).

Compound **7b**: 15.04 (59), 35.59 (14.8), 49.95 (100), 51.96 (36.5), 121.96 (53.2) and 210.85 (20.4); FAB: 330.72 (M.Wt. – Cl).

Compound **7c**: 63.93 (20), 77.9 (33.8), 105.87 (47.8), 121.85 (36.5), 134.81(23.4), 210.85 (100) and 256.67 (32).

Compound **7d**: 41.99 (53.5), 50.97 (31.8), 77.94 (100), 105.87 (78), 121.85 (58.5), 134.9 (96.5) and 210.85 (50.9).

Biological screening

A-Pharmacological screening

Analgesic activity[25]

Male adult albino mice (20-28g) were divided into six 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 acacia. The reaction time was evaluated directly before and after ½, 1, 2, 3 and 5 hours of compounds administration. Results of analgesic activity of the test compounds and 5-ASA are listed in Tables 8 and 9.

Table 8: The analgesic activity of some 3-N-(4`-hydroxy-3`-substituted phenyl)carbamoyl-1-methylpyridinium iodides (compds. **5b-d** and **f**) on heat–induced pain in mice.

Compd. No.	R	The average reaction time (second) at different times after compound administration				
-	-	½ hr	1 hr	2 hr	3 hr	5 hr
Control	-	8.6±0.74	8.1±0.74	9±1	8±1.29	7±0.16
5b	OCH_3	15.87±0**	28.8±2.3**	40.6±2.2**	52.6±1.9**	25.2±0.9**
5c	OC_2H_5	13.8±0.8**	14.7±0.7**	19.5±1.1**	18.2±1.72**	17 ±1.48**
5d	OC_3H_7	14.3±0.9**	15±1.3**	18.3±1.7**	21.32±1.7**	13.1± 0.7**
5f	$NHCH_3$	19.2±1.8**	18.25±1**	24.5±0.9**	24.25±0.9**	11.5±0.4*
5-ASA	-	12.5±1.1**	23.12±2**	31.87±2.5**	45.37 ±1.4**	9.14±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.

Table 9: The analgesic activity of some 3-carbamoyl-1-(N-(4`-hydroxy-3`-substituted phenyl) carbamoyl)methylpyridinium chlorides on heat-induced pain in mice.

Compd. No.	R	The average reaction time (second) at different times after compound administration				
-	-	½ hr	1 hr	2 hr	3 hr	5 hr
Control	-	8.6±0.74	8.1±0.74	9±1	8±1.29	7±0.16
7b	OCH_3	14.3±0.1**	19.2±1.8**	26.7±2.2**	44.8±1**	33±1.5**
7f	$NHCH_3$	18.9±1.4**	19±1.1**	22.5±2.1**	25.2±1*	21±1.9**
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.

Anti-inflammatory activity[26]

Male adult albino rats (180-200 g) were divided into seven groups, each of five animals. Solutions or suspensions of the test compounds and reference drugs in 5% gum acacia were injected 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 ½, 1, 2, 3 and 5 hours after carrageenin administration.

Results of anti-inflammatory evaluation of the test compounds and reference drugs are listed in Tables 10 and 11.

Table 10: The anti-inflammatory activity of some 3-N-(4`-hydroxy-3` substituted phenyl carbamoyl-1-methylpyridinium iodides against carrageenin–induced rat paw edema.

Compd. No.	R	Volume of the right paw (mm) at different times after carrageenin injection (time in hours)					
-	-	0	½	1	2	3	5
Control	-	3.1±0.02	5.9±0.18	6.3±0.19	6.4±0.18	6.9±0.18	7±0.16
5b	OCH_3	3.02±0.03	4.8 ±0.25*	4.4±0.18**	4.1±0.09**	4.3±0.43**	5±0.3**
5c	OC_2H_5	3.1±0.03	4.9±0.22*	4.8±0.16**	4.8±0.2**	4.9±0.34**	5.8±0.2**
5d	OC_3H_7	3.06±0.02	4.7±0.15**	4.6±0.1**	4.6±0.19**	4.3±0.19**	5.6±0.18**
5f	$NHCH_3$	3.01±0.01	4.6 ±0.12**	4.8±0.25**	4.9±0.4**	5±0.27**	5.1±0.2**
5-ASA	-	3.05±0.01	4.3 ±0.19**	4.1±0.09**	4.2±0.25**	4.4±0.18**	6.2±0.75
SASP	-	2.99±0.02	4.7±0.12**	4.7±0.12**	4.5±0**	4.7±0.18**	5.7±0.29**

- 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 intraperitoneally into rats (10 mg/kg) 30 minutes before carrageenin injection.

Table 11: The anti-inflammatory activity of some 3-carbamoyl-1-(N-(4`-hydroxy-3`-substituted phenyl)carbamoyl)methylpyridinium chlorides gainst carrageenin –induced rat paw odema.

Compd. No.	R	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
7b	OCH_3	3.02±0.03	4.9±0.33*	4.7 ±0.15**	4.5**±0.2	4.4±0.2**	5.5±0.1**
7f	$NHCH_3$	3.1±0.03	5±0.31	4.6±0.18**	4.9** ±0.4	5.5±0.4**	5.9±0.4*
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.

Gastric ulcerogenic effect[27,28]

Male adult albino rats (120-200 g) were divided into seven groups each of five animals. Animals were starved but had free access to water for 24 hours before the experiment. The 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 hours, 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 compound **5b** was compared with that of aspirin.

Determination of acute toxicity (LD_{50})[29]

Groups of male adult albino mice, each of five animals (25-30 g), were injected i.p. with graded doses of compound **5b**. The percentage of mortality, in each group of animals, was determined, 72 hr latter to injection. Computation of LD_{50} was processed by a graphical method.

B- Hydrolytic cleavage of 5-ASA prodrugs by intestinal microflora

***In vitro* study**[11]

Incubation of **5b**, 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 isotonic phosphate buffer pH 6.8 corresponding to 65 mg of 5-ASA was added. The mixture was incubated at 37° 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 plate was visualized by UV at 254 nm.

Bacteriological testing[30]

Streptococcus pyogenus cultivated on blood agar medium was further cultivated in cooked meat medium[31] (½ g in 5 mL brain heart infusion) by incubation for 48 hr. 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**[11]

Release of 5-ASA and N-acetyl-5ASA in feces after oral administration of **5b** or sulfasalazine: Male Sprague-Dawely rats, three for each compound, weighing (120-150 g), were housed in individual metabolic cages and starved for 24 hour 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. The mixture was vortexed and centrifuged at 8000 r.p.m. Aliquots of the clear supernatants were examined and detected by TLC as mentioned above.

pH stability of compound 5b[11]

Chemical stability of compound 5b was determined by preparing solutions of this compound (140 mg/mL) in phosphate buffer (pH 6.8) and in hydrochloric acid buffer (pH 1.2) then incubating the solutions at 37° for 6 h. At one-hour time intervals, aliquots of the solutions were taken and examined by TLC for the released 5-ASA and the intact compound as previously described.

RESULTS AND DISCUSSION

Chemistry

The synthetic pathways are shown in scheme 1. Methyl, ethyl and propyl esters (**3b-d**) have been prepared by direct esterification of 5-ASA with the appropriate alcohol in presence of conc. H_2SO_4 in an approximate 60% yield. Propyl ester is liquid and there are no physical or spectral data found in the available literatures for it. Therefore, it is identified by i.r. and ^{1}H-NMR spectral data (Table 1). 5-Aminosalicylamides (**3e-j**) were prepared from the corresponding salicylamides (**1e-j**), in an analogy to a reported method by coupling with phenyldiazonium chloride followed by reductive splitting of the resulting azo compounds (**2e-j**).[32]

Although the prepared amides (**3e-j**) were reported in patents, but their physical and spectral data are not found in the available literature.[24,33] The i.r. spectra of (**3e-j**) showed the presence of strong broad band at about 1640 cm^{-1} which is attributed to amidic carbonyl function and NH bending.. In addition to (O-H) stretching at about 3485 cm^{-1} and (N-H) stretching at about 3225 cm^{-1}. ^{1}H-NMR spectra showed broad singlet integrating for two protons corresponding to the introduced amino group.

5-(Nicotinoyl)aminosalicylic acid esters and amides (**4b-j**) were prepared by reacting nicotinic acid with thionyl chloride and the in situ formed nicotinoyl chloride was allowed to react with the appropriate amine in boiling toluene in presence of pyridine in an analogy to a reported method.[34]

Quaternization of compounds (**4b-j**) with methyl iodide (2 equivalents) gave good yields (78-90%) of quaternary compounds (**5b-j**). In general, the prepared pyridinium salts (**5b-j**) are crystalline solids and most of them are hygroscopic. Some of the compounds were isolated as hydrates, which is in agreement with the known tendency of pyridinium salts to crystallize as hydrates.[35]

The ^{1}H-NMR spectra of compounds **5b-d** showed singlet at δ= 9.65 - 10 ppm due to C2-H of the pyridinium ring and a remarkable downfield singlet at δ= 4.70 ppm integrated for three protons due to (N^+CH_3) indicated the occurrence of quaternization. Compounds 5b-d were subjected to Electron impact (EI) mass spectrometry, while **5c** was subjected to Fast Atomic Bombardment (FAB) using glycerol as matrix. In EI the molecular ion peak [$M^{\cdot+}$] was not detected, this was expected because of the ionic nature of the quaternary compounds. The FAB spectra showed ($M^{\cdot+}$– I) as the base peak in most cases.

Compounds (**6 a-j**) were prepared through the reaction of compounds (**3a-j**) with chloroacetyl chloride in benzene. The ^{1}H-NMR spectra of compounds (**6a-j**) showed singlet at the range δ= 4.20–4.40 ppm integrating for two protons of ($COCH_2Cl$).

Quaternization of nicotinamide compounds (**6a-j**) in acetonitrile provided the final compounds (**7a-j**). The ^{1}H-NMR spectra of pyridinium compounds **7a-j** showed singlet at δ= 9.65-10 ppm due to C2-H of the pyridinium ring and characteristic signal at δ= 5-6 ppm due to (CH_2) indicating the occurrence of quaternization. Compounds **7b-d** were subjected to EI mass spectrometry, the cationic entity was either not detected or very weakly (about 0.2%) appeared. Thus, the other technique Fast Atomic Bombardment (FAB) was utilized using glycerol as matrix. In FAB the cation entities were detected in high intesties (about 80-100%).

Structures of all intermediates have been confirmed by i.r. and p.m.r. spectral data, while those of the final compounds by microanalyses, i.r., p.m.r. and some by mass spectrometry as shown in Tables (1-7).

Biological screening

A-Pharmacological screening

Six of the synthesized compounds (**5b-d**, **5f**, **7b** and **7f**) were, preliminary, tested for their analgesic and antiinflammatory activity:

The analgesic activity was determined by the hot plate method[25] using 5-ASA as standard. Results are listed in Tables 8, 9.

The antiinflammatory activity was evaluated in rats by carrageenin-induced inflammation[26] using 5-ASA and sulfasalazine as standard drugs. Results are recorded in Tables 10 and 11. Results obtained were statistically evaluated to determine their significance.

Study of data recorded in Tables (8-11) revealed that methyl esters (**5b** and **7b**) are more effective in both tests than the

corresponding N-methylamides (**5f** and **7f**). Also, the methyl ester (**5b**) of the trigonelline series is more effective than that of the nicotinamide series (**7b**) in the range of 3 hr after administration as an analgesic, while compound (**7b**) was more active than (**5b**) after 5 hr of administration in this respect (Tables 8 and 9).

Also, comparison of the analgesic activity of esters (**5b-d**) (Table 8) showed that the analgesic activity is diminished as the alkyl group of the ester moiety is increased in bulk than methyl. The same sequence was also noticed for these compounds (**5b-d**) as antiinflammatories (Table 10). This result may be attributed to the fact that the methyl ester may fit better to the corresponding receptor involved in the pharmacodynamic phase of these compounds either as analgesics, or as antiinflammatories. Amides (**5f** and **7f**) showed comparable analgesic and antiiflammatory effects (Tables 8-11).

It is noteworthy to mention that compound (**5b**) exhibited superior analgesic activity than 5-ASA at all time intervals after administration (Table 8). Moreover, it showed more potent anti-inflammatory effect than sulfasalazine at 1,2, 3 and 5 hours after administration and similar effects at zero and half hour (Table 10). Accordingly compound (**5b**) was subjected for study of its ulcerogenic effect and determination of its LD_{50}.

Ulcerogenic effect

Gastric ulcerogenic effect was determined in rats for compound **5b** by reported method[27,28] and showed no gastric ulcerations (zero% lesions), while aspirin induced 80% gastric lesions. These results are consistent with the known beneficial effects of 5-ASA and its derivatives on gastrointestinal tract inflammation[37] along with promotion of endogenous cytoprotective prostaglandins.[37]

Acute toxicity (LD_{50})

LD_{50} of **5b** was found to be 375 mg/kg (i.p.), while those of 5-ASA is (469 mg/kg i.p.)[29] and aspirin is (533 mg/kg i.p.).[38]

B- Hydrolytic cleavage of 5-ASA prodrugs by intestinal microflora

The role of gut flora in drug metabolism[5] 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 simulate gut environment with regard to the pH and redox potential. Evidence for metabolism by the intestinal microflora *in vivo* usually relies on the detection of a specific microbial drug metabolites.

In the present work, qualitative determination of the metabolic outcome of compound (**5b**) has been done in rats in comparison to sulfasalazine by *in vivo* and *in vitro* studies.[11,30] The study involved the ability of release of 5-ASA as a hydrolytic product under the effect of microflora.

***In vitro* study**

a- Release of 5-ASA after incubation of 5b with cecal and colonic contents of rats

When **5b** was incubated with the cecal contents, 5-ASA was released similarly to that released from sulfasalazine and was detected by TLC (Table 12). 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.

b- Bacteriological study

It has been reported that the predominant intestinal microflora are those of the non-sporing, strictly, anaerobes.[5] In this work, analysis of incubation mixtures (compound **5b** with Streptococcus pyogenus in cooked meat medium) showed considerable disappearance of the test prodrugs together with the appearance of the released 5-ASA as indicated by TLC (Table 12). This finding indicates that both ester and amide linkages of the test compounds are able to hydrolyse by the intestinal microflora.[5]

***In vivo* study**

Recovery of 5-ASA and N-acetyl-5-ASA in the feces after oral administration of **5b** 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 prodrugs **5b** in addition

to trace amounts of intact **5b** as indicated by their R_f values after visualization by UV at 254 nm as shown in Table (12).

Table 12: R_f values of compound (**5b**) and reference standards.

Compd.	5-ASA	N-acetyl-5-ASA	**5b**	SASP
R_f	0.63	0.80	0.27	0.85

C- pH stability of compound 5b[11]

pH stability of **5b** was determined to confirm that the release of 5-ASA is due to bacterial metabolism and 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 **5b** is chemically stable during its transit through the gastrointestinal tract to the colon, similar results have been, previously, reported for salicylamide and its derivatives.[39]

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.

Acknowledgement

The authors are greatly indebted to Prof. A. F. Abdel-Ghaffar, vice president of El-Minia University and Prof. A. O. Abdel-Zaher, Dept. of Pharmacology, Faculty of Medicine, University of Assiut for their kind supervision and sincere help during performing the bacteriological and pharmacological screenings.

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