Synthesis and Pharmacological Evaluation of Some Dual-Acting Amino-alcohol Ester Derivatives of Flurbiprofen and 2-[1,1′-Biphenyl-4-yl]acetic Acid: A Potential Approach to Reduce Local Gastrointestinal Toxicity

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The search for safer non-steroidal anti-inflammatory drugs (NSAIDs) continues with the failure of anticipated 'ideal' anti-inflammatory agents, the coxibs, on long-term usage. Increased gastric motility and acidity due to the free carboxy group are involved in the etiology of gastric toxicity, common to conventional NSAIDs. Keeping this fact in mind, it was planned to modify some of the conventional NSAIDs to amino-alcohol ester derivatives, which satisfied the structural requirements for these compounds to possess anticholinergic activity in the intact form. Besides blocking the acidic carboxylic group, incorporation of anticholinergic acivity in these molecules was expected to reduce the gastric toxicity by decreasing gastric acid secretion and motility. Synthesis and pharmacological evaluation of six different *N*,*N*-disubstituted amino-ethyl ester derivatives, structurally resembling the amino-alcohol ester class of anticholinergic agents, each for [1,1′-biphenyl]-4-acetic acid (**3**) and flurbiprofen (**10**), have been reported as potential substitutes for these NSAIDs, with improved therapeutic profile. All the ester derivatives were found to have sufficient chemical stability in buffers (pH 2.0 and 7.4), ensuring them to be absorbed as intact moieties from the gastrointestinal tract. A significant reduction in ulcerogenic potency in comparison to the parent drugs with a slightly higher anti-inflammatory potency suggests that the majority of these candidates have an improved therapeutic profile over their parent drugs. Hence, a promising novel approach, different from the conventional prodrug concept, has been successfully worked out to overcome the local gastric toxicity, yielding therapeutically better compounds for long-term oral anti-inflammatory therapy.

1. Introduction. – Recent withdrawals of selective cyclooxygenase-2 (COX-2) inhibitors (rofecoxib) from the market have belied the hope of coxibs being 'ideal' anti-inflammatory agents because of their significant adverse cardiovascular effects [1][2]. Looking at the withdrawal of coxibs, and the time and cost involved in developing an entirely new drug molecule, it seems to be a safer and lucrative approach to modify the existing, well-established, time tested conventional non-steroidal anti-inflammatory drugs (NSAIDs) in order to reduce/get rid of their toxic/side effects.

The clinical utility of the conventional acidic NSAIDs continues to be principally limited by their undesired side effects mainly on the gastrointestinal (GI) tract. Researchers have indicated that the main causes of NSAID-induced gastropathy are reduced mucosal prostaglandin (PG) levels, increased gastric acidity, and increased gastric motility. The increased gastric motility leads to a reduced mucosal blood flow, hypoxia, and destruction of the mucous bicarbonate barrier, which prevents back-

diffusion of pepsin and hydrogen ions from lumen into the mucosal layer [3]. Moreover, microcirculation of gastroduodenal mucosa supplies energy and oxygen to mucosal cells, removes hydrogen ions, waste products, and transports bicarbonate to the surface of the gastric epithelium. In this way, the mucosal blood flow plays a very crucial role in supporting the defense mechanism of mucosa [4]. All common NSAIDs, because of their acidic nature, have a local action ('direct contact effect') on gastric epithelium; in the acidic gastric medium, their ionization get suppressed, and, thus, they are easily absorbed locally as non-ionized species. Intracellularly (where the pH is higher than that in gastric lumen), they are extensively ionized and get entrapped, expressing their anti-PG action at the site even more intensely and lead to further mucosal damage [5][6]. Hence, the direct contact effect is a combination of local irritation produced by the free carboxylic acid group of NSAIDs and the local inhibition of cytoprotective actions of PG on gastric mucosa [7]. The prodrug approach involves preparation of a pharmacologically inactive derivative of the active drug that liberates the active parent moiety after entering the circulation. The use of simple ester and amide prodrugs to temporarily mask the acidic group of NSAIDs has remained a very widely exploited approach to decrease local GI toxicity due to the direct contact effect [8][9]. Unlike the parent drugs, these prodrugs do not inhibit the COX enzyme throughout their GI transit and thereby overcome the local PG inhibition exhibited by the parent drugs. These prodrugs thus possess the potential to avoid parent NSAIDs-mediated direct GI damage, while maintaining the efficacy *via* the systemic action of the regenerated active drug.

The approach reported herein to reduce GI toxicity of acidic NSAIDs is innovative and novel, and has additional benefits over the usual prodrug approach. In this approach, it was planned to convert the carboxylic group (present in NSAIDs) into *N*,*N*-disubstituted 2-aminoethyl esters. Such a conversion would serve dual purpose. First, it would block the acidic carboxylic group like any other simple ester (prodrug approach). Second, such a derivatization would incorporate an entirely new pharmacodynamic property into the original NSAIDs molecules, as these designed molecules would have structural resemblance with 'amino-alcohol ester' class of anticholinergic drugs (*e.g.*, cyclopentolate (=2-(dimethylamino)ethyl α-(1-hydroxycyclopentyl)benzeneacetate; **1**)). They are expected to possess anticholinergic activity in the intact form.

N,*N*-Disubstituted amino-alcohol esters of these NSAIDs (*i.e.*, of **3** and **10**) were designed specifically to make them structurally resemble the amino-alcohol ester class of anticholinergics. A terminal tertiary 'nitrogen' atom with an $-CH_2CH_2-$ bridge between this terminal N-atom and the ester C=O group, and the presence of bulky groups at the C=O terminal are the structural features of amino-alcohol ester class of anticholinergics [10]. These structural requirements are satisfied by these designed amino-alcohol esters of NSAIDs. The designed amino-alcohol esters would possess certain advantages over the reported prodrugs (simple esters) of NSAIDs. In the first place, the designed amino esters would block the free carboxylic group of NSAIDs like any other ester/amide prodrug. Second, the amino-ester derivatives will be in protonated form (as salts of amines at the tertiary N-atom) in the acidic pH of stomach with a blocked carboxy group. So, there would be neither any local absorption (due to the ionic form) nor any local gastric irritation (due to the absence of a free

carboxy group). And, when they enter the alkaline pH of the intestine, the amino esters would exist as unionized (lipophilic) species thus facilitating their absorption. After absorption into systemic circulation the amino esters would be cleaved enzymatically/non-enzymatically to release the parent NSAIDs, to elicit the desired anti-inflammatory effect. So, besides preventing local GI irritation by temporarily blocking the carboxy group present in the NSAIDs, the existence of anticholinergic activity in the intact esters would further aid in reducing the GI toxicity by *i*) decreasing gastric acid secretions and *ii*) decreasing gastric motility to maintain optimal mucosal blood flow. Hence, the amino esters would possess the anticholinergic protection only at the desired site, the gut, before absorption, and, after absorption into the circulation, they would be cleaved by the ubiquitous esterases. A fast cleavage following the absorption would be a further asset for these amino esters, since they would be devoid of any anticholinergic action after entering the circulation, and this would ensure them to be free from systemic anticholinergic side-effects like dryness of mouth.

It was anticipated that the combination of these two properties, of being anticholinergic when intact and anti-inflammatory after hydrolysis, might lead to a much wider scope for these esters with additional indications in ophthalmic inflammatory conditions and in conditions of bronchospasm, where there is a demand for both anticholinergic and anti-inflammatory activities.

In this paper, synthesis and evaluation of dual-acting esters of [1,1′-biphenyl]-4-acetic acid (**3**) and flurbiprofen (=2-fluoro-α-methyl[1,1′-biphenyl]-4-acetic acid; **10**) are reported for reducing local GI toxicity, which is associated with the parent moieties. Compound **3**, an active metabolite of fenbufen (=γ-oxo-[1,1′-biphenyl]-4-butanoic acid; **2**), shows a three times higher anti-inflammatory activity than the parent drug but is also considerably more ulcerogenic than fenbufen [11].

2. Results and Discussion. – 2.1. *Synthesis.* Compound **3** was synthesized according to the procedure reported in [12]. The ester derivatives **4**–**9** and **11**–**16** were synthesized successfully in good yields (*Fig. 1*). Treatment of **3** and **10** with $SOCl_2$ yielded the corresponding acid chlorides, which, on further treatment with an appropriate amino alcohol under basic conditions, followed by simple workup, gave the oily esters. The esters were converted to the corresponding hydrochloride salts in the presence of dry HCl. The salt forms made the purification and handling of the compounds easy. Hence, the synthetic methodology involved a single derivatization to yield solid HCl salts of the esters in pure form. The structures of all the compounds were confirmed by their spectral and elemental analyses.

2.2. *Hydrolyses.* The chemical and enzymatic hydrolyses of all the synthesized esters were carried out in aqueous buffer solutions (pH 2.0 and 7.4) and in human serum (80%), respectively. UV-Spectrophotometric methods, specific for the estimation of the released parent drugs, **3** and **10**, even in the presence of the intact amino ester derivatives **4**–**9** and **11**–**16**, were developed. In buffer of pH 2.0, all the ester derivatives were found to be intact during 8 h with no observable hydrolysis. Based on these observations, it could be concluded that the stomach would not be exposed to the free carboxylic group of the NSAIDs, as the amino esters (*i.e.*, **4**–**9** and **11**–**16**) would not be cleaved during their stay in the stomach. In acidic gastric medium, the unionized forms of common NSAIDs are easily absorbed into the cells and get entrapped

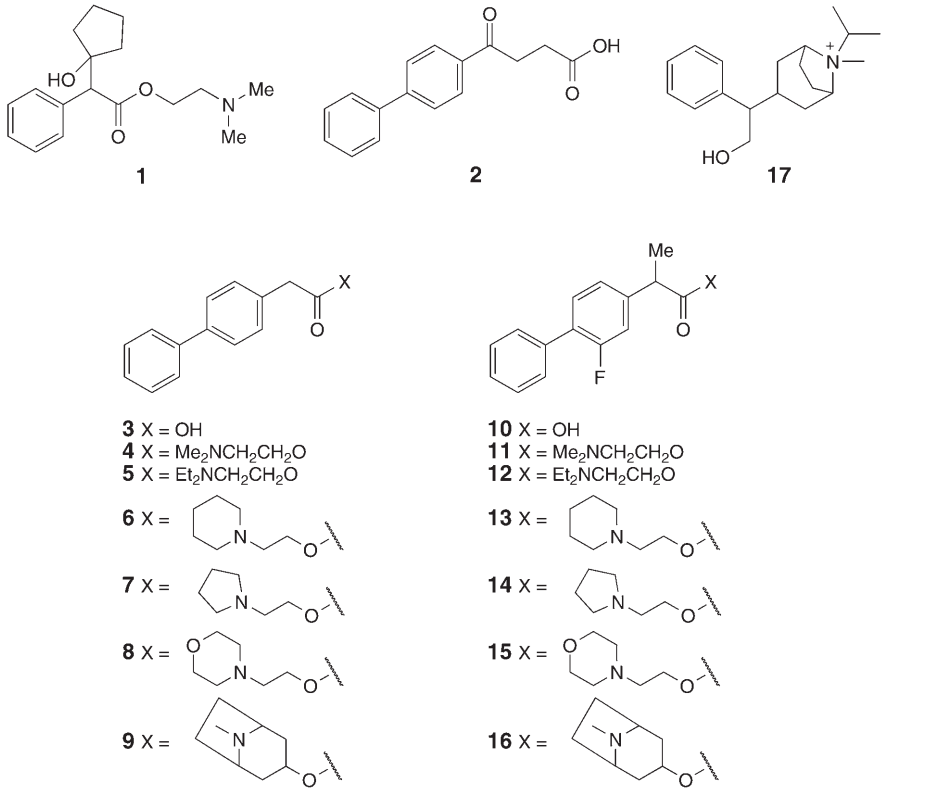

Fig. 1. *Structures of cyclopentolate* (**1**)*, fenbufen* (**2**)*, the parent drugs* **3** and **10***, synthesized amino-alcohol esters* **4**–**9** and **11**–**16**, and *ipratropium* (**17**)

intracellularly as ionized species due to higher intracellular pH. This entrapment results in more intense localized anti-prostaglandin action and serious mucosal damage [6][7]. However, the designed esters existing in the polar protonated ionic forms at the acidic pH of stomach would resist the absorption into the gastric cells. So, there is no question of intracellular entrapment of the compounds and, therefore, the local PG inhibition. At pH 7.4, compounds **4**–**8** and **11**–**15** showed a very low rate of hydrolysis and large half-lives (46 to 491 h, *Table 1*), while compounds **9** and **16** did not get hydrolyzed during the observation period. It was expected of these compounds to be absorbed in intact form from the intestine during this period. It could be expected from this data that the non-ionic form of the esters existing in the alkaline pH of the intestine would be absorbed intact and enter the circulation. All the derivatives except **9** and **16** exhibited enzymatic hydrolysis in 80% human serum. It can be concluded from these observations that these derivatives would survive the GI conditions successfully and release the parent moiety in the blood stream after absorption by the action of the serum esterases. Compounds **9** and **16** showed resistance towards chemical and enzymatic hydrolysis due to the presence of a bulky secondary alcohol-ester moiety.

Chemical stability at pH 2.0 and 7.4, and the enzymatic susceptibility (esterases present in serum) of derivatives **4**–**8** and **11**–**15** would enable these compounds to show an anticholinergic (protective) effect (decreased gastric secretions and motility) only at the desired site, *i.e.*, the gut, and be free of any systemic anticholinergic side effects. After entering the circulation, enzymatic cleavage of the amino-alcohol esters would liberate the parent NSAIDs for eliciting the desired anti-inflammatory effect. For this reason, these derivatives can be administered orally for the systemic anti-inflammatory actions, because they would be lacking the typical side effects of anticholinergic agents like dryness of mouth.

Table 1. *Chemical and Enzymatic Hydrolysis Data of Compounds* **4**–**9** *and* **11**–**16**

Compound	$t_{1/2}$ [h][a]) (pH 7.4)	Percent release of parent drug[a]) (80% human serum)		
		½ h	1 h	2 h
4	46	27.3	40.5	81.3
5	52	11.4	15.5	43.4
6	68	4.0	11.6	31.6
7	64	10.7	11.7	23.2
8	139	3.2	11.9	15.6
9	NH[b])	NH[b])	NH[b])	NH[b])
11	121	13.2	41.9	51.1
12	153	17.1	17.2	43.9
13	505	15.2	19.6	31.9
14	250	11.8	15.4	29.8
15	491	4.5	8.1	17.9
16	NH[b])	NH[b])	NH[b])	NH[b])

[a]) Studies in triplicate at $37 \pm 1°$. [b]) No observable hydrolysis up to a period of 8 h in buffer and up to 2 h in 80% human serum.

2.3. *Pharmacological Evaluation*. All of the ester derivatives showed anticholinergic activity, though much weaker than the standard anticholinergic drug, atropine. The pA_2 values (*Table 2*) ranged from 4.70 to 6.31 with maximum anticholinergic activity residing in the tropinol ester derivatives **9** and **16**. But it may be noted that the total quantum of anticholinergic activity would be substantial due to the high doses of these esters which are governed by the therapeutic doses of the parent NSAIDs.

In carrageenan-induced rat hind paw edema model for acute anti-inflammatory activity, except for the tropinol esters **9** and **16**, all the compounds exhibited either higher or comparable activity with respect to the parent drugs at equimolar dose levels (*Table 2*; *Fig. 2*). The tropinol esters **9** and **16** did not exhibit any anti-inflammatory effect even when administered at 100 mg/kg body weight. Absence of an anti-inflammatory effect for the tropinol esters could be correlated to their resistance to chemical or enzymatic hydrolysis to release the parent drugs (*i.e.*, **3** and **10**). Higher percentage of the anti-inflammatory effect observed for the other ester derivatives might be the result of either better absorption of the esters from the GI tract resulting in higher bioavailability of the compounds or due to the higher selectivity of these derivatives towards the COX-2 enzyme than the parent NSAIDs. It is reported [13] that

Table 2. *Biological Activities of Parent Drugs and Their Amino-alcohol Esters*

Compound	pA_2[a])	Paw volume [ml][b]) (% Inhibition)	Ulcer index
Control	–	0.92 ± 0.02 (0)	0.00
3	–	0.35 ± 0.07 (61)	0.56 ± 0.07
(Dose)[c])		0.04	2.05
4	4.70	0.20 ± 0.03 (78)[d])	0.23 ± 0.02[d])
5	5.10	0.19 ± 0.07 (78)[d])	0.28 ± 0.03[d])
6	5.39	0.14 ± 0.03 (85)[d])	0.26 ± 0.05[d])
7	4.84	0.26 ± 0.09 (72)[d])	0.18 ± 0.05[d])
8	5.21	0.18 ± 0.05 (80)[d])	0.16 ± 0.04[d])
9	6.08	0.91 ± 0.02 (0)[d])	0.00
10	–	0.13 ± 0.02 (85)	0.80 ± 0.05
(Dose)[c])		0.03	0.93
11	4.89	0.12 ± 0.03 (87)	0.23 ± 0.04[d])
12	4.73	0.11 ± 0.01 (88)	0.14 ± 0.02[d])
13	5.76	0.10 ± 0.02 (89)	0.26 ± 0.06[d])
14	4.88	0.19 ± 0.06 (79)	0.17 ± 0.09[d])
15	5.79	0.12 ± 0.02 (87)	0.13 ± 0.03[d])
16	6.31	0.93 ± 0.04 (0)	0.00

[a]) In our laboratory conditions, the pA_2 value for atropine was found to be 8.20. [b]) Anti-inflammatory data are given as paw volume + standard error of mean, and values in parentheses indicate % inhibition of edema formation. [c]) The parent compounds **3** and **10** were dosed p.o. on mmol kg^{-1} body weight. Test compounds were dosed equimolar to their respective parent drugs. [d]) $p < 0.05$ *vs.* parent drug. The control group received only the vehicle.

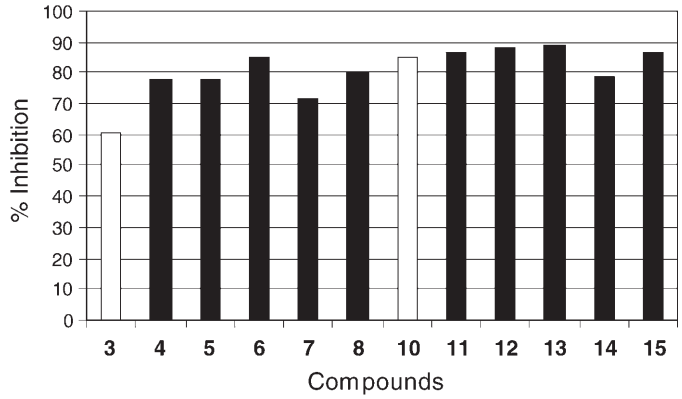

Fig. 2. *Inhibition* [%] *of rat paw edema by the parent NSAIDs and their derivatives*

selectivity towards the COX-2 enzyme increased in some ester and amide derivatives of NSAIDs.

The derivatives **4**–**8** and **11**–**15** evaluated for their ulcerogenic potential showed a significant reduction (*Table 2*) in their ulcerogenicity at equimolar doses with respect to their corresponding parent drugs. The stability of the derivatives in buffers of pH 2.0 and 7.4 indicated that the derivatives would be absorbed intact without exposing the

gastrointestinal tract to the acidic carboxy group. Hence, the observed residual ulcerogenicity of the derivatives has been attributed to the systemic inhibition of prostaglandins by the parent drugs, which were released upon hydrolysis of the esters in circulation. The absence of any ulcers in case of hydrolysis-resistant tropinol esters **9** and **16** supported this assumption.

Anticholinergic agents (ipratropium (**17**), tiatropium) are used with β-agonists for inflammatory disorders like chronic obstructive pulmonary diseases. An interesting observation made for the tropinol esters regarding their resistance to chemical and enzymatic hydrolyses along with high anticholinergic potency reveals them to be probable candidates for treatment in such respiratory disorders. In such conditions, the tropinol esters can be administered by inhalation, and, sooner or later, when they are hydrolyzed in lungs to release the parent moiety, the resulting anti-inflammatory action might also prove to be beneficial. Similarly, the tropinol esters could also have ophthalmic use. NSAIDs have found less use in ophthalmic inflammatory conditions due to their highly polar nature in the alkaline pH of eye leading to poor absorption. These tropinol derivatives would be in non-ionic form in the slightly alkaline pH of the eye and are expected to be absorbed easily, and the inherent anticholinergic activity (mydriasis) present in them could aid in ophthalmic disc examination, with the normal anti-inflammatory activity after cleavage to the parent drug by the esterases.

3. Conclusions. – Chemical modification of the two NSAIDs **3** and **10** into the *N*,*N*-disubstituted amino-alcohol ester derivatives have resulted in moieties with superior gastric tolerability in comparison to their respective parent drugs. Hydrolysis studies have revealed that they possess desired physicochemical properties (good aqueous stability and high enzymatic conversion) to be regarded as derivatives useful for oral administration.

Apart from showing a significant reduction in ulcerogenic potency, all the compounds have retained the desired pharmacodynamic properties except for the tropinol esters **9** and **16**. The existence of anticholinergic activity in these derivatives would contribute in reducing the gastric irritation by decreasing the gastric acid secretion as well as the gastric motility, thereby aiding in maintaining normal mucosal blood blow. Moreover, the metabolism pattern for these derivatives would remain more or less the same as the respective parent drugs. Good aqueous solubility of the hydrochloride salt of these esters could also be exploited in formulating parenteral and ophthalmic dosage forms of these NSAIDs.

It is worth mentioning that the synthesized ester derivatives cannot be designated as prodrugs, since they possess the intrinsic anticholinergic activity prior to being hydrolyzed to the respective parent NSAIDs.

In brief, a novel concept has been worked out, which is somewhat different from the conventional prodrug approach, to reduce the local GI toxicity of NSAIDs. This approach has resulted in better substitutes of the parent drugs for long-term oral anti-inflammatory therapy. It may be noted that this approach may be the rarest of the rare examples in the history of drug research where a compound in intact form possesses one type of favorable pharmacological activity and a different but desirable pharmacological property on metabolism.

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Experimental Part

General. Parent drug **10** was obtained from *M/s Knoll Pharmaceutical Ltd*, India, as a gift sample. *Lambda* (Type IV) carrageenan and the various amino alcohols were purchased from *Sigma-Aldrich*. Tropinol was obtained by the basic hydrolysis (MeOH/KOH) of atropine. Dist. H_2O was used in preparation of buffer solns. Anh. Na_2SO_4 was used as drying agent. M.p.: in open capillaries; uncorrected. UV Spectra: *Shimadzu UV-1601* spectrophotometer; λ_{max} in nm. IR Spectra: *Shimadzu-8300 FTIR* using KBr disc; in cm^{-1}. ^{1}H-NMR Spectra: 300-MHz instrument for solns. in $CDCl_3$; δ in ppm.

General Procedure for the Preparation of N,N-*Disubstituted Amino-alcohol Esters (HCl Salts) of* **3** *and* **10**. A mixture of parent drug **3** (1.9 g, 9 mmol) and $SOCl_2$ (2.0 ml, 27 mmol) in dry toluene (5 ml) was heated at 80° for 2 h under dry conditions. Excess of $SOCl_2$ and the solvent were removed under vacuum to obtain the oily acid chloride. The acid chloride was dissolved in freshly distilled $CHCl_3$ (30 ml), and anh. K_2CO_3 (3.0 g) was added. The appropriate *N*,*N*-disubstituted amino alcohol (27 mmol) was dissolved in freshly distilled $CHCl_3$ (10 ml) and added dropwise to the well-stirred acid chloride soln. at r.t., followed by refluxing in a water bath for 3 h. The mixture was further diluted with $CHCl_3$ (200 ml) and filtered. The org. layer was washed with ice-cold H_2O to remove the excess of the amino alcohol, dried (Na_2SO_4), and the solvent was removed under vacuum to give an oily product. The oily product was dissolved in dry (i-Pr)$_2$O, and dry HCl was passed through the soln. to give a white solid precipitate of the ester hydrochloride. The salt so obtained was filtered and crystallized from acetone/(i-Pr)$_2$O. The compounds **4**–**9** so obtained were characterized by physical, spectroscopic, and elemental data. Compounds **11**–**16** were also synthesized by this procedure starting from the parent drug **10**.

2-(Dimethylamino)ethyl [1,1′-biphenyl]-4-acetate Hydrochloride (**4**). Yield: 47.8%. M.p. 167–169°. UV (MeOH): 253 (4.41). IR: 1743 (C=O). ^{1}H-NMR: 2.75 (*s*, 2 Me_2N); 3.33 (*t*, CH_2N); 3.79 (*s*, ArCH_2); 4.56 (*t*, CH_2O); 7.33–7.63 (*m*, 9 arom. H); 12.75 (br. *s*, HCl). Anal. calc. for $C_{18}H_{22}ClNO_2$: C 67.60, H 6.93, N 4.38; found: C 67.32, H 6.72, N 4.51.

2-(Diethylamino)ethyl [1,1′-biphenyl]-4-acetate Hydrochloride (**5**). Yield: 85.4%. M.p. 152–153°. UV (MeOH): 253 (4.31). IR: 1749 (C=O). ^{1}H-NMR: 1.30 (*t*, (*Me*$CH_2)_2N$); 3.02 (*q*, MeCH_2); 3.26 (*t*, CH_2N); 3.75 (*s*, ArCH_2); 4.63 (*t*, CH_2O); 7.27–7.59 (*m*, 9 arom. H); 12.55 (br. *s*, HCl). Anal. calc. for $C_{20}H_{26}ClNO_2$: C 69.05, H 7.53, N 4.03; found C 68.87, H 7.22, N 4.27.

2-(Piperidin-1-yl)ethyl [1,1′-biphenyl]-4-acetate Hydrochloride (**6**). Yield: 31.9%. M.p. 161–163°. UV (MeOH): 253 (4.35). IR: 1743 (C=O). ^{1}H-NMR: 1.25–3.38 (*m*, CH_2N, $CH_2(2)$, $CH_2(3)$, $CH_2(4)$, $CH_2(5)$, $CH_2(6)$); 3.74 (*s*, ArCH_2); 4.65 (*t*, CH_2O); 7.33–7.597 (*m*, 9 arom. H); 12.20 (br. *s*, HCl). Anal. calc. for $C_{21}H_{25}ClNO_2$: C 70.28, H 7.02, N 3.90; found: C 69.61, H 6.89, N 4.12.

2-(Pyrrolidin-1-yl)ethyl [1,1′-biphenyl]-4-acetate Hydrochloride (**7**). Yield: 67.5%. M.p. 138–140°. UV (MeOH): 253 (4.39). IR: 1743 (C=O). ^{1}H-NMR: 2.02–3.28 (*m*, CH_2N, $CH_2(2)$, $CH_2(3)$, $CH_2(4)$, $CH_2(5)$); 3.74 (*s*, ArCH_2); 4.65 (*t*, CH_2O); 7.30–7.60 (*m*, 9 arom. H); 12.20 (br. *s*, HCl). Anal. calc. for $C_{20}H_{24}ClNO_2$: C 69.45, H 6.99, N 4.05; found: C 69.82, H 7.25, N 4.36.

2-(Morpholin-4-yl)ethyl [1,1′-biphenyl]-4-acetate Hydrochloride (**8**). Yield: 43.0%. M.p. 150–152°. UV (MeOH): 253 (4.28). IR: 1743 (C=O). ^{1}H-NMR: 2.64–3.21 (*m*, $CH_2N(CH_2)_2$); 3.79 (*s*, ArCH_2); 3.80–4.17 (*m*, $CH_2(2)$, $CH_2(6)$); 4.68 (*t*, CH_2O); 7.33–7.58 (*m*, 9 arom. H); 13.50 (br. *s*, HCl). Anal. calc. for $C_{20}H_{24}ClNO_3$: C 66.38, H 6.69, N 3.87; found: C 66.61, H 6.46, N 3.52.

8-Methyl-8-azabicyclo[3.2.1]oct-3-yl [1,1′-biphenyl]-4-acetate Hydrochloride (**9**). Yield: 54.4%. M.p. 256–258°. UV (MeOH): 253 (4.34). IR: 1727 (C=O). ^{1}H-NMR: 1.86–1.94 (*m*, $CH_2(6)$, $CH_2(7)$, and $CH_2(4)$ or $CH_2(2)$); 2.65 (*s*, MeN); 2.75–3.00 (*m*, H–C(1), H–C(5)); 3.49–3.60 (*m*, $CH_2(2)$ or $CH_2(4)$); 3.68 (*s*, ArCH_2); 5.15 (*t*, H–C(3)); 7.31–7.60 (*m*, 9 arom. H). Anal. calc. for $C_{19}H_{25}ClNO_2$: C 68.15, H 7.53, N 4.18; found: C 68.48, H 7.72, N 4.29.

2-(Dimethylamino)ethyl 2-(2-Fluoro[1,1′-biphenyl-4-yl])propanoate Hydrochloride (**11**). Yield: 32.7%. M.p. 120–122°. UV (MeOH): 247 (4.46). IR: 1743 (C=O). ^{1}H-NMR: 1.55 (*d*, Me); 2.72 (*s*, Me_2N); 3.27 (*t*, CH_2N); 3.88 (*q*, ArC*H*); 4.55 (*m*, CH_2O); 7.09–7.53 (*m*, 8 arom. H). Anal. calc. for $C_{19}H_{23}ClFNO_2$: C 64.86, H 6.59, N 3.98; found: C 64.69, H 6.91, N 4.27.

2-(Diethylamino)ethyl 2-(2-Fluoro[1,1′-biphenyl-4-yl])propanoate Hydrochloride (**12**). Yield: 27.9%. M.p. 115–118°. UV (MeOH): 247 (4.30). IR: 1732 (C=O). ^{1}H-NMR: 1.28 (*t*, (*Me*CH$_2$)$_2$N); 1.55 (*d*, Me); 3.01 (*q*, (MeC*H*$_2$)$_2$N); 3.26 (*t*, CH$_2$N); 3.82 (*q*, ArC*H*); 4.60 (*m*, CH$_2$O); 7.08–7.53 (*m*, 8 arom. H). Anal. calc. for $C_{21}H_{27}ClFNO_2$: C 66.39, H 7.16, N 3.69; found: C 66.57, H 7.42, N 3.81.

2-(Piperidin-1-yl)ethyl 2-(2-Fluoro[1,1′-biphenyl-4-yl])propanoate Hydrochloride (**13**). Yield: 44.7%. M.p. 153–154°. UV (MeOH): 247 (4.34). IR: 1736 (C=O). ^{1}H-NMR: 1.54 (*d*, Me); 1.69–3.33 (*m*, CH$_2$N, CH$_2$(2), CH$_2$(3), CH$_2$(4), CH$_2$(5), CH$_2$(6)); 3.83 (*q*, ArC*H*); 4.64 (*m*, CH$_2$O); 7.08–7.53 (*m*, 8 arom. H). Anal. calc. for $C_{22}H_{27}ClFNO_2$: C 67.42, H 6.94, N 3.57; found: C 67.29, H 6.66, N 3.82.

2-(Pyrrolidin-1-yl)ethyl 2-(2-Fluoro[1,1′-biphenyl-4-yl])propanoate Hydrochloride (**14**). Yield: 29.3%. M.p. 95–96°. UV (MeOH): 247 (4.35). IR: 1733 (C=O). ^{1}H-NMR: 1.54 (*d*, Me); 2.02–3.33 (*m*, CH$_2$N, CH$_2$(2), CH$_2$(3), CH$_2$(4), CH$_2$(5)); 3.86 (*q*, ArC*H*); 4.58 (*m*, CH$_2$O); 7.08–7.53 (*m*, 8 arom. H). Anal. calc. for $C_{21}H_{25}ClFNO_2$: C 66.75, H 6.67, N 3.71; found: C 66.55, H 6.39, N 3.95.

2-(Morpholin-4-yl)ethyl 2-(2-Fluoro[1,1′-biphenyl-4-yl])propanoate Hydrochloride (**15**). Yield: 52.6%. M.p. 118–120°. UV (MeOH): 247 (4.28). IR: 1740 (C=O). ^{1}H-NMR: 1.54 (*d*, Me); 2.58–3.29 (*m*, CH$_2$N, CH$_2$(3), CH$_2$(5)); 3.75–4.20 (*m*, ArC*H*, CH$_2$(2), CH$_2$(6)); 4.66 (*m*, CH$_2$O); 7.07–7.52 (*m*, 8 arom. H); 13.3 (br. *s*, HCl). Anal. calc. for $C_{21}H_{25}ClFNO_3$: C 64.04, H 6.40, N 3.56; found: C 63.83, H 6.59, N 3.72.

8-Methyl-8-azabicyclo[3.2.1]oct-3-yl 2-(2-Fluoro[1,1′-biphenyl-4-yl])propanoate Hydrochloride (**16**). Yield: 39.9%. M.p. 196–198°. UV (MeOH): 247 (4.31). IR: 1727 (C=O). ^{1}H-NMR: 1.53 (*d*, Me); 1.85–1.96 (*m*, CH$_2$(6), CH$_2$(7)); 2.04–2.09 (*m*, CH$_2$, CH$_2$(4)); 2.64 (*s*, MeN); 2.99–3.13 (*m*, H–C(1), H–C(5)); 3.55–3.65 (*m*, CH$_2$(2) or CH$_2$(4)); 3.69–3.76 (*m*, MeC*H*); 5.14 (*t*, H–C(3)); 7.07–7.53 (*m*, 8 arom. H); 12.51 (br. *s*, HCl). Anal. calc. for $C_{20}H_{27}ClFNO_2$: C 65.30, H 7.40, N 3.81; found: C 65.57, H 7.21, N 3.69.

Hydrolyses. Chemical Stability of Compounds **4**–**9** *in Aqueous Systems.* Reactions were initiated by maintaining a 1-mg/ml concentration of the esters **4**–**9** in buffers (pH 2.0 and 7.4) at 37±1°. At definite time intervals, a sample (1.0 ml) was withdrawn and transferred to a separating funnel containing buffer of pH 2.0 (9 ml). This acidified soln. was extracted into $CHCl_3$ (2×5 ml). The combined $CHCl_3$ extract was further extracted into NaOH (0.1N, 2×5 ml). The pooled aq. extract was heated on a water bath for 30 min, cooled to r.t., and the absorbance was measured for the combined aq. layer at 255 nm (λ_{max}) using a blank treated similarly but without the drug soln. The rate constants, *k*, for the hydrolysis of the compounds were determined by linear regression of the plot of log of residual ester *vs.* time. Triplicate samples were analyzed, and $t_{1/2}$ was calculated ($t_{1/2}$ 0.693/*k*; *Table 1*).

Enzymatic Susceptibility of Compounds **4**–**9** *in 80% Human Serum.* Pooled human serum (4 ml) was taken in a stoppered conical flask and maintained at 37±1° in a water bath. To this, the soln. of **4**–**9** (1 ml, 5 mg/ml in pH 7.4 phosphate buffer) was added, and, at appropriate time intervals, aliquots (0.5 ml) were withdrawn and transferred to a separating funnel containing Cl_3CCOOH (1 ml, 10% (*w*/*v*)). Further, 8.5 ml of buffer (pH 2.0) was added, and this protein precipitated suspension was extracted with (i-Pr)$_2$O (2×5 ml), and the combined ethereal extract was then extracted with NaOH soln. (0.1N, 2×5 ml). The pooled aq. extract in each case was heated in a water bath for 30 min, cooled to r.t., and the absorbance was measured at 255 nm against blank NaOH soln. (0.1N), which was treated similarly but without the drug soln. The percent release of the parent drug over definite time interval was calculated. Three determinations were performed, and average percentage release of the parent drug was calculated (*Table 1*).

Chemical Stability of Compounds **11**–**16** *in Aqueous Systems.* Reactions were initiated by maintaining a 1-mg/ml concentration of the esters **11**–**16** in buffers (pH 2.0 and 7.4) at 37±1°. At definite time intervals, a sample (1.0 ml) was withdrawn and transferred to a separating funnel containing buffer of pH 2.0 (9 ml). This acidified soln. was extracted into hexane (2×5 ml). The combined org. layer was dried, and absorbance was measured at 237 nm (λ_{max}) against a blank (hexane), which was treated similarly. The hydrolysis rate constants, *k*, and $t_{1/2}$ were calculated as for compounds **4**–**9** (*Table 1*).

Enzymatic Susceptibility of Compounds **11**–**16** *in 80% Human Serum.* The procedure followed was similar to that for compounds **4**–**9** in 80% human serum to obtain the protein precipitated suspension which was extracted into hexane (2×5 ml). The combined org. layer was dried, and absorbance was measured at 237 nm against a blank (hexane). The percent release of the parent drug over definite time

interval was calculated. Three determinations were performed, and the average percentage release of the parent drug was calculated (*Table 1*).

Pharmacological Evaluation. The parent drugs **3** and **10** as standard reference drugs were given orally, suspended in 1% (carboxymethyl)cellulose in normal saline. The ester derivatives were administered orally as solns. in normal saline at dose levels equimolar to their respective parent compounds. The data obtained in the pharmacological experiments were subjected to statistical analysis using *the Student*'s *t* test, and the chosen level of significance was $p < 0.05$. The protocol for the animal experiments performed was approved by the IAEC (Institutional Animal Ethics Committee) as registered under CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Government of India.

Anticholinergic Activity. The anticholinergic activity was determined on isolated rat ileum [14]. Rats weighing 150–200 g housed single were fasted overnight having access to water. The abdomen was dissected, and the ileum was placed in *Tyrode* soln. at $37 \pm 1°$ with aeration. A tissue of 1.0–1.5 cm length was mounted under tension of 1 g. The tissue was stabilized with washings of fresh *Tyrode* every 10 min, and the dose response curve (DRC) with acetylcholine was recorded until a maximum response was reached. The tissue was then allowed to be in contact with the *Tyrode* containing either the ester derivatives (*i.e.*, **4**–**9** and **11**–**16**) or atropine as a standard anticholinergic drug for 1/2 h, and the DRC was repeated for acetylcholine. The percent response was calculated for both the DRCs and plotted against log [M] of acetylcholine on the same graph paper to find the EC_{50} in presence and absence of the antagonist (amino-ester derivative or atropine). The pA_2 value was calculated according to the formula $pA_2 = -\log[M] + \log(x-1)$ where, [M] = molar concentration of antagonist and $x = EC_{50}$ found in presence of antagonist/EC_{50} found in absence of antagonist.

Anti-Inflammatory Activity. Anti-inflammatory activity for the derivatives was determined by carrageenan-induced rat hind paw edema assay [15]. Rats of either sex in the weight range of 150–200 g were divided into groups of six each and were fasted for 24 h prior to administration of the compounds. The paw volume was determined by plethysmometer (*UGO-Basil*, Italy) at 0 and 3 h after carrageenan injection (0.1 ml of 1% (*w*/*v*) in normal saline) in the subplantar region of left hind paw. The ester hydrochlorides **4**–**9** were dosed equivalent to 0.04 mmol kg^{-1} body weight of compound **3**. Similarly, the derivatives **11**–**16** were given at a dose equivalent to 0.03 mmol kg^{-1} body weight of drug **10**. The control group received only the vehicle. Results (*Table 2*) are expressed as percentage inhibition of edema formation, calculated by the formula: % Inhibition of paw edema $= (1 - Ed_{drug}/Ed_{control}) \times 100$, Ed_{drug} and $Ed_{control}$ are the edema volumes in drug treated and control groups, resp.

Ulcerogenicity. Sprague–Dawley rats ($n = 6$, 150–200 g) of either sex were used. The rats were fasted for 36 h with water *ad libitum* prior to administration of drug solns. and for 4 h post dosing [16]. The control group received no drug. Compound **3** was administered p.o. 2.05 mmol kg^{-1} body weight, and the derivatives **4**–**9** at dose equivalent to 2.05 mmol kg^{-1} body weight of **3**. The animals were sacrificed, and their stomach were dissected out, cut along the greater curvature, washed with normal saline, and kept in 5% formalin for 15 min, and gastric mucosa was observed for the lesions using a 2×2 binocular magnifier. The results were expressed as ulcer index as per the formula: *Ulcer index* $= 10(A_u/A_m)$, where A_m = total mucosal area, $A_u = A_l + A_c + A_p$, A_l is area of linear lesions, A_c is area of circular lesions, and A_p is total number of petechiae/5. The derivatives of parent drug **10** were also evaluated similarly at a dose equivalent to 0.93 mmol kg^{-1} of the parent drug **10**.

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