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Synthesis of new chemical entities from paracetamol and NSAIDs with improved pharmacodynamic profile

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Abstract—It was envisaged to combine high antipyretic activity of paracetamol into commonly used NSAIDs. To achieve this goal new chemical entities were synthesized by chemically combining paracetamol and NSAIDs, and biologically evaluated for their antipyretic, analgesic, anti-inflammatory and ulcerogenic potential. The acid chloride of parent NSAIDs was reacted with excess of *p*-aminophenol to yield the desired *p*-amidophenol derivatives (**1B**–**7B**). Acetate derivatives (**1C**–**7C**) of these phenols (**1B**–**7B**) were also prepared by their treatment with acetic anhydride, in order to see the impact of blocking the free phenolic group on the biological activity of the derivatives. All the synthesized *p*-amidophenol derivatives showed improved antipyretic activity than paracetamol with retention of anti-inflammatory activity of their parent NSAIDs. These compounds elicited no ulcerogenicity unlike their parent drugs.

1. Introduction

Paracetamol is a commonly used analgesic and antipyretic drug. However, it has only weak anti-inflammatory effects. The failure of paracetamol to exert anti-inflammatory activity may be attributed to the fact that paracetamol is only a weak inhibitor of COX in the presence of high concentration of the peroxides that are found in the inflammatory lesions.[1] In contrast, its antipyretic effect may be explained by its ability to inhibit COX in the brain, where peroxide tone is low.[2] Single or repeated therapeutic doses of paracetamol have no effect on the cardiovascular and respiratory system nor does the drug produce gastric irritation/erosion. Recent reports[3,4] suggest that overdose of paracetamol is the most common cause of acute liver failure (ALF) in United States and United Kingdom.

Nonsteroidal anti-inflammatory agents (NSAIDs) are the most commonly prescribed drugs in the world but, their use as anti-inflammatory, antipyretic, antithrombotic and analgesic agents continues to be limited due to their undesired side effects mainly on the gastrointestinal (GI) tract. NSAIDs are known to have inhibitory activity for both the isoforms of the cyclooxygenase (COX) enzyme. They vary considerably in their tendency to cause gastric erosions and ulcers.[1] Gastric damage by these agents is caused by at least two distinct mechanisms. One is by inhibiting the cytoprotective COX-1 in the stomach and second by physical contact and ion-trapping mechanism.[5,6] The use of prodrugs to temporarily mask acidic group of NSAIDs has been postulated as an approach to decrease the GI toxicity due to direct contact effect.[7,8] These prodrugs release the parent moieties after absorption by undergoing enzymatic/chemical hydrolysis. On the other hand selective inhibition of the inducible COX-2 enzyme, sparing the constitutive COX-1, formed the basis for designing of COXIBs with minimum degree of ulcerogenic risk. This new concept of treating inflammation related disease came into effect with the consecutive launch of celecoxib[9] and rofecoxib.[10,11]

Willoughby and colleagues[12] described the effect of some selective COX-2 inhibitors and some dual (COX-1 and COX-2) inhibitors on carrageenan pleurisy in the rat over a time period ranging from 0 to 48 h after injection of the irritant. This investigation gave surprising results. The COX-2 inhibitors showed anti-inflammatory activity early in the inflammatory response, coincident with the expression

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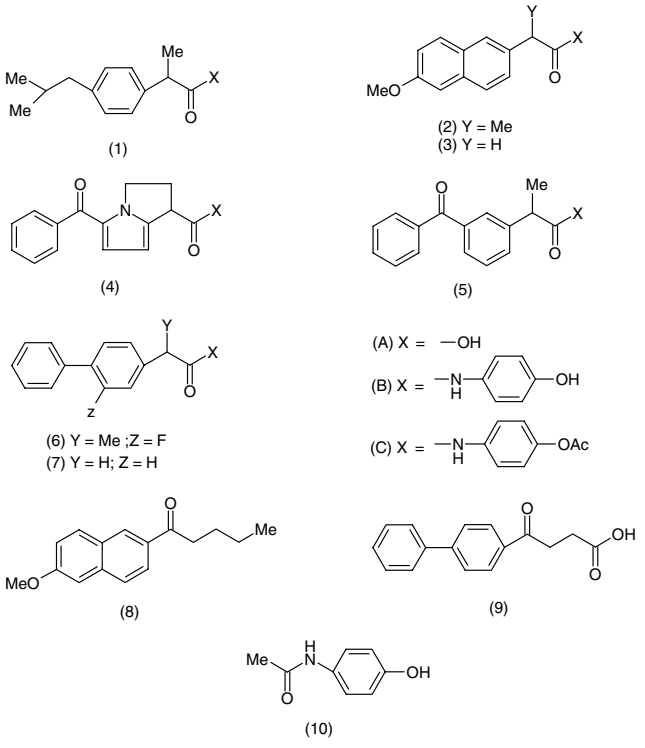
of COX-2 protein as did the older dual inhibitors such as indomethacin. However, by 6 h the COX-2 inhibitors were without effect although the dual inhibitors still showed efficacy. At this point the COX-2 protein as shown by Western blotting was no longer present. This study showed the supremacy of conventional NSAIDs over the COXIBs for chronic use. Moreover, withdrawal of rofecoxib from market recently by its originators due to adverse cardiovascular effects puts a big question mark on the safety profile of other COXIBs in the long-term therapy.

It has been reported[13] that conversion of the carboxylic group containing NSAIDs to ester and amide functions makes them more selective towards COX-2 enzyme. Taking the above said report and the potential cardiovascular dangers posed by COXIBs into cognizance, and the cost and time involved in the discovery of a new drug, it was thought of converting some common NSAIDs into *p*-amidophenol derivatives. *p*-Aminophenol has been evaluated in the past[1] as analgesic–antipyretic but its N-acetylated derivative, that is, paracetamol (**10**) has been found to be the most suitable, therapeutically. It was planned to substitute the acetyl group in paracetamol (**10**) with the carboxylic group containing NSAIDs as the acyl groups. Threefold advantages were visualized through such chemical conversion. First, the free carboxylic group present in the NSAIDs would be blocked by a nonhydrolysable (at physiological pH) amide linkage thereby preventing the local contact mechanism, which was partially responsible for GIT ulceration by these NSAIDs. Second, these new derivatives are expected to show more selectivity towards COX-2 enzyme as reported[13] for the amide derivatives for NSAIDs, resulting in further reduction in ulcerogenicity of the parent NSAIDs. Finally, due to the structural resemblance of these amides with paracetamol they are expected to exhibit substantial antipyretic effect like the parent drug paracetamol (**10**).



2. Results and discussion

2.1. Chemistry

Commonly used carboxylic group containing NSAIDs like ibuprofen (**1A**) naproxen (**2A**), 6-methoxy-2-naphthylacetic acid (6-MNA) (**3A**) (active metabolite of nabumetone (**8**)), ketorolac (**4A**), ketoprofen (**5A**), flurbiprofen (**6A**) and biphenylacetic acid (**7A**) (active metabolite of fenbufen (**9**)) were chosen for this study. These compounds were converted into the acid chlorides by treatment with thionyl chloride under anhydrous conditions. The acid chloride was reacted with excess of *p*-aminophenol in dioxane to yield a mixture containing the desired *p*-amidophenol derivatives (**1B**–**7B**) and the undesired 4-aminophenyl esters. The unwanted 4-aminophenyl esters were removed by washing the chloroform solution of the mixture continuously with dilute hydrochloric acid until it became free from it. The products so obtained were purified by repeated recrystallizations. All the synthesized *p*-amidophenol derivatives were converted into their acetates in order to see the impact of blocking the free phenolic group on the biological activity of the derivatives. The acetate derivatives (**1C**–**7C**) were prepared by treatment of the free phenolic compounds (**1B**–**7B**) with acetic anhydride in pyridine. The synthesized compounds conform to the assigned structures, as deduced on the basis of their spectral and elemental data. Biphenylacetic acid (**7A**) was prepared by Friedel–Crafts acetylation of biphenyl followed by Kindler-modified Willgerodt reaction of the 4-acetyl derivative.[14] Similarly, 6-MNA (**3A**) was obtained by carrying out Friedel–Crafts acetylation of nerolin[15] and then submitting the product so obtained to Kindler-modified Willgerodt reaction to obtain the desired product (**3A**).[16]

2.2. Biological

The compounds (**1B**–**7C**) were synthesized with a view to incorporate the antipyretic activity component of paracetamol (**10**) into the NSAIDs with their normal anti-inflammatory activity but without GIT ulceration. So, all the synthesized derivatives (**1B**–**7C**) were evaluated for their antipyretic activity in animal model[17] using lipopolysaccharide (LPS) (from *Escherichia coli*) endotoxin for producing pyrexia. Percent reversal of the body temperature (antipyretic activity) was determined (Table 1) using paracetamol (**10**) as the standard antipyretic drug. Interesting results were obtained as shown in Figure 1. All the compounds with free phenolic group have shown superior antipyretic activity than paracetamol (**10**). It may be noted that this activity was determined on equal weight bases with paracetamol for all the compounds. That means the *p*-amidophenol component in the derivatives (**1B**–**7C**) on molar basis is much less than that present in paracetamol (**10**). But, free phenolic group seems to be essential in these compounds also, like paracetamol (**10**) for superior antipyretic activity as the acylation of the phenolic hydroxyl group has led to decrease in antipyretic activity.

COX-2 inhibiting activity for the synthesized compounds was determined using the Cayman COX-2

Table 1. Biological activities of synthesized compounds

Compound	% inhibition of COX-2 at 22 μM concn	Anti-inflammatory activity		Ulcerogenic potential		Analgesic activity		Antipyretic activity	
		Dose mg/kg	% inhibition	Dose mg/kg	Ulcer index	Dose mg/kg	% inhibition	Dose mg/kg	Pyrexia % reversal of body temperature
1A	8.4	20	60.0	200	0.5013 ± 0.021	20	41.9	—	ND
1B	10.4	28.8	34.8	290	Nil	28.8	19.1	25	87.4
								10	49.3
1C	NI	32.9	38.7	330	Nil	32.9	36.5	25	75.0
								10	43.5
2A	81.8	20	81.1	200	0.981 ± 0.038	20	43.0	—	ND
2B	12.7	27.9	39.4	280	Nil	27.9	20.7	25	59.5
								10	25.7
2C	NI	31.6	27.6	316	Nil	31.6	21.8	25	12.5
3A	10.5	20	55.4	200	0.630 ± 0.052	20	42.2	—	ND
3B	8.9	28.4	44.6	284	Nil	28.4	30.8	25	75.0
3C	NI	32.3	41.5	323	Nil	32.3	28.5	25	68.7
4A	100	10	75.0	75	0.484 ± 0.030	20	54.1	—	ND
4B	31.8	13.5	86.4	101.3	Nil	25	52.6	25	55.8
4C	11.1	15.2	83.5	114	Nil	30.4	58.2	25	37.5
5A	100	20	86.7	200	0.822 ± 0.039	25	74.8		ND
5C	4.3	29.5	72.3	295	Nil	36.9	68.4	25	50.0
6A	15.4	8	84.0	25	0.80 ± 0.048	10	54.9	—	ND
6B	12.3	11	68.9	34.4	Nil	13.1	45.8	25	58.5
6C	NI	12.4	58.1	38.8	Nil	15.5	52.3	25	18.5
7A	10.5	10	60.9	500	0.564 ± 0.069	25	51.7	—	ND
7B	8.9	14.3	57.9	715	Nil	35.8	47.8	25	82.4
7C	NI	16.3	59.6	815	Nil	40.8	45.2	25	67.5
								10	35.5
10	—	—	—	—	—	—	—	25	63.8
								10	39.9

NI, no inhibition at the test dose; ND, not determined.

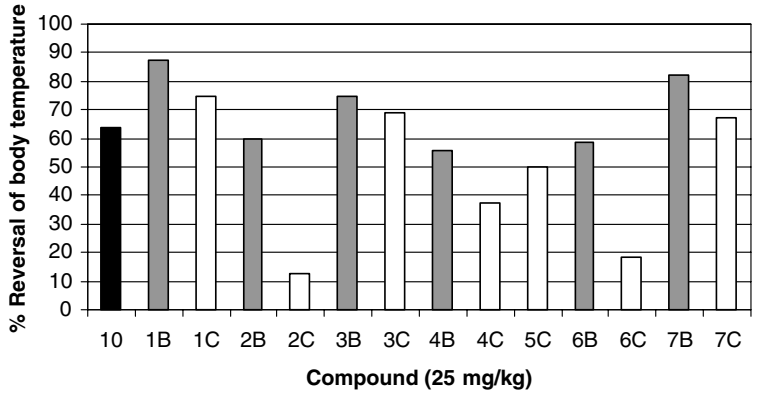


Figure 1. Antipyretic activity of synthesized compounds in rats.

colorimetric screening kit.[18] No uniform conclusion could be drawn on the basis of this study except that all the acetylated derivatives showed no inhibition at the test dose level. So, it was planned to perform anti-inflammatory activity in the in vivo model using carrageenan-induced paw edema.[19] The activity was carried out on equimolar basis to the parent drug. The results of the study (Table 1) indicate that the conversion of free carboxylic group in the NSAIDs to the amide linkage caused reduction in the anti-inflammatory activity of the parent drugs, in general. However, ketorolac (derivative **4B**) was found to be an exception where an increase in inflammatory activity was noticed. Acetylation of free phenolic group was observed not to change this activity drastically. Since NSAIDs do possess peripheral analgesic activity, the synthesized derivatives were evaluated for this activity using the writhing method[20] in mice. No straightforward conclusion could be drawn from this study except that all the synthesized derivatives possessed analgesic activity with somewhat less potency than their parent NSAIDs.

Ulcerogenic potential of the synthesized compounds was determined in rat model.[21] It was very encouraging to note that none of the compounds showed any ulcer formation in the test animals. This may be due to the dual factors of blocking of the free acidic group and more selectivity of these compounds to inhibit COX-2.

3. Conclusion

Conversion of the conventional carboxylic group containing NSAIDs into *p*-amidophenol derivatives has resulted into new potential drugs having much improved antipyretic activity. These compounds also possessed sufficiently good potency to be used as anti-inflammatory drugs with nil ulcerogenicity in acute model of this biological testing. Keeping in view the therapeutic superiority of the classical NSAIDs over COXIBs on longer duration of usage, newer potential compounds have been developed possessing high analgesic–antipyretic activity with nil/minimum GIT ulceration. Acetylation of free phenolic group in these synthesized compounds was not found to be a suitable proposition as it decreased the potency.

4. Experimental

4.1. Chemistry

Melting points were determined using a heating block-type melting point apparatus and are uncorrected. Purity of the compounds was ascertained by thin-layer chromatography (TLC) on silica gel plates (60 F_{254}; Merck), visualizing with ultraviolet light or iodine vapors. The yields reported here are unoptimized. IR spectra were recorded using KBr disc method on a Shimadzu FT-IR Model 8300. ^{1}H NMR spectra on a Brucker 300 MHz spectrometer were recorded in $CDCl_3$ (chemical shifts in δ ppm). Assignment of exchangeable protons (N*H*) was confirmed by D_2O exchange studies. Elemental analyses were obtained on Carlo Erba, Italy, and Perkin-Elmer instruments. All the compounds were purified by recrystallization from acetone–pet. ether.

4.2. General procedure for the preparation of compounds 1B–7B

4.2.1. Representative preparation of 4-[2-(4-isobutylphenyl)propionamido]phenol (1B). Ibuprofen **1A** (2 g) was dissolved in dry toluene (25 ml) and thionyl chloride (2 ml) was added dropwise with constant stirring. The reaction mixture was heated at 80 °C on a water bath for 2 h, and excess thionyl chloride and toluene was removed under vacuum. The residue so obtained was dissolved in dry dioxane (25 ml) and added dropwise to a solution of *p*-aminophenol (5 g) in dioxane (50 ml) with stirring. The reaction mixture was stirred for 1 h at room temperature and heated on water bath for 3 h. Excess of dioxane was removed under vacuum, the reaction mixture acidified with dilute hydrochloric acid and extracted with chloroform (3× 50 ml). The combined organic extract was washed successively with hydrochloric acid (5%) until free from the basic impurities, dried over sodium sulfate and solvent removed. The residue so obtained was purified by crystallization from acetone–pet. ether. Yield 50%; mp 112–115 °C; IR (KBr): 3299, 1653, 1536, 1510, 1234, 827 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.90 (d, 6H), 1.59 (d, 3H), 1.85 (m, 1H), 2.45 (d, 2H), 3.68–3.71 (q, 1H), 6.95 (br s, 1H), 6.68–6.73 (m, 2H), 7.10–7.15 (m, 4H), 7.28–7.31 (m, 2H). Anal. Calcd for $C_{19}H_{23}NO_2$: C, 76.74; H, 7.80; N, 4.71. Found: C, 76.48; H, 7.53; N, 4.38.

4.2.2. 4-[2-(6-Methoxy-2-naphthyl)propionamido]phenol (2B). Yield 35%; mp 156–159 °C; IR (KBr): 3327, 1652, 1539, 1512, 1226, 826 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.60 (d, 3H), 3.86–3.89 (q, 1H), 3.91 (s, 3H), 8.90 (br s, 1H), 6.65–6.69 (m, 2H), 7.10–7.15 (m, 1H), 7.32–7.55 (m, 4H), 7.69–7.77 (m, 3H). Anal. Calcd for $C_{20}H_{19}NO_3$: C, 74.75; H, 5.96; N, 4.36. Found: C, 74.94; H, 6.19; N, 4.52.

4.2.3. 4-[2-(6-Methoxy-2-naphthyl)acetamido]phenol (3B). Yield 38%; mp 201–203 °C; IR (KBr): 3285, 1657, 1539, 1512, 825 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.68 (s, 2H), 3.85 (s, 3H), 6.64–6.67 (m, 2H), 7.10–7.14 (m, 1H), 7.33–7.43 (m, 4H), 7.70–7.77 (m, 3H), 9.15 (br s, 1H), 9.92 (br s, 1H). Anal. Calcd for $C_{19}H_{17}NO_3$: C, 74.25; H, 5.58; N, 4.56. Found: C, 73.91; H, 5.24; N, 4.28.

4.2.4. 5-Benzoyl-*N*-(4-hydroxyphenyl)-2,3-dihydro-1*H*-pyrrolizine-1-carboxamide (4B). Yield 43%; mp 228–229 °C; IR (KBr): 3300, 1661, 1651, 1513, 1240, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.81 (m, 2H), 4.07 (m, 1H), 4.52 (m, 2H), 6.09 (br s, 1H), 6.64–6.68 (m, 2H), 6.89–6.90 (d, 1H), 7.44–7.57 (m, 6H), 7.81–7.85 (m, 2H). Anal. Calcd for $C_{21}H_{18}N_2O_3$: C, 72.82; H, 5.24; N, 8.09. Found: C, 72.99; H, 5.52; N, 8.27.

4.2.5. 4-[2-(3-Benzoylphenyl)propionamido]phenol (5B). Sticky material. Yield 47%; IR (KBr): 3286, 1652, 1535, 1510, 834 cm^{-1}.

4.2.6. 4-[2-(2-Fluoro-4-biphenyl)propionamido]phenol (6B). Yield 40%; mp 170–173 °C; IR (KBr): 3291, 1652, 1539, 1531, 1237, 827 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.61 (d, 3H), 3.68–3.71 (q, 1H), 6.97 (br s, 1H), 6.74–6.77 (m, 2H), 7.16–7.23 (m, 2H), 7.28–7.47 (m, 6H), 7.53–7.55 (m, 2H). Anal. Calcd for $C_{21}H_{18}FNO_2$: C, 75.21; H, 5.41; N, 4.18. Found: C, 75.66; H, 5.22; N, 4.37.

4.2.7. 4-[2-(4-Biphenyl)acetamido]phenol (7B). Yield 15%; mp 205–208 °C; IR (KBr): 3246, 1651, 1546, 1514, 1245, 749 cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 3.68 (s, 2H), 7.30 (br s, 1H), 6.72–6.75 (m, 2H), 732–7.45 (m, 7H), 7.53–7.58 (m, 4H), 9.24 (br s, 1H). Anal. Calcd for $C_{20}H_{17}NO_2$: C, 79.19; H, 5.65; N, 4.62. Found: C, 79.49; H, 5.83; N, 4.38.

4.3. General procedure for the preparation of compounds 1C–7C

4.3.1. Representative preparation of 4-[2-(4-isobutylphenyl)propionamido]phenyl acetate (1C). Compound **1B** (0.4 g) was dissolved in dry pyridine (1 ml) and cooled in ice bath. Acetic anhydride (1.0 ml) was added dropwise with stirring. Reaction mixture was heated on water bath for 3 h and allowed to cool to room temperature. It was poured over crushed ice containing conc hydrochloric acid (3 ml). The solid was filtered, dried and recrystallized from acetone–pet. ether. Yield 85%; mp 145–147 °C; IR (KBr): 3354, 1734, 1684, 1541, 1510, 839 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.90 (d, 6H), 1.59 (d, 3H), 1.85 (m, 1H), 2.26 (s, 3H), 2.46 (d, 2H),

3.67–3.70 (q, 1H), 7.21 (br s, 1H), 6.96–7.01 (m, 2H), 7.10–7.16 (m, 4H), 7.39–7.44 (m, 2H). Anal. Calcd for $C_{21}H_{25}NO_3$: C, 74.31; H, 7.42; N, 4.13. Found: C, 74.68; H, 7.21; N, 4.01.

4.3.2. 4-[2-(6-Methoxy-2-naphthyl)propionamido]phenyl acetate (2C). Yield 90%; mp 212–216 °C; IR (KBr): 3380, 1748, 1668, 1528, 1502, 857 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.60 (d, 3H), 2.25 (s, 3H), 3.86–3.89 (q, 1H), 3.91 (s, 3H), 8.97 (br s, 1H), 6.95–7.00 (m, 2H), 7.09–7.14 (m, 1H), 7.38–7.54 (m, 4H), 7.66–7.78 (m, 3H). Anal. Calcd for $C_{22}H_{21}NO_4$: C, 72.71; H, 5.82; N, 3.85. Found: C, 72.52; H, 5.62; N, 3.67.

4.3.3. 4-[2-(6-Methoxy-2-naphthyl)acetamido]phenyl acetate (3C). Yield 84%; mp 175–176 °C; IR (KBr): 3305, 1750, 1651, 1539, 1506, 839 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.27 (s, 3H), 3.67 (s, 2H), 3.85 (s, 3H), 6.99–7.03 (m, 2H), 7.11–7.15 (m, 1H), 7.33–7.47 (m, 4H), 7.70–7.77 (m, 3H) 9.06 (br s, 1H). Anal. Calcd for $C_{21}H_{19}NO_4$: C, 72.19; H, 5.48; N, 4.01. Found: C, 72.49; H, 5.64; N, 3.88.

4.3.4. *N*-(4-Acetoxyphenyl)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxamide (4C). Yield 89%; mp 197–198 °C; IR (KBr): 3260, 1755, 1661, 1539, 1508, 720 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.28 (s, 3H), 2.80 (m, 2H), 4.08 (m, 1H), 4.53 (m, 2H), 6.17 (br s, 1H), 6.89–6.90 (d, 1H), 7.04–7.06 (m, 2H), 7.45–7.57 (m, 6H), 7.82–7.85 (m, 2H). Anal. Calcd for $C_{23}H_{20}N_2O_4$: C, 71.12; H, 5.19; N, 7.21. Found: C, 71.48; H, 5.41; N, 7.52.

4.3.5. 4-[2-(3-Benzoylphenyl)propionamido]phenyl acetate (5C). Yield 49%; mp 87–88 °C; IR (KBr): 3357, 1730, 1683, 1661, 1538, 1508, 838 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.62 (d, 3H), 2.27 (s, 3H), 3.72–3.76 (q, 1H), 7.18 (br s, 1H), 6.98–7.02 (m, 2H), 7.44–7.52 (m, 5H), 7.57–7.65 (m, 2H), 7.68–7.72 (m, 1H), 7.78–7.82 (m, 3H). Anal. Calcd for $C_{23}H_{21}NO_4$: C, 73.58; H, 5.64; N, 3.73. Found: C, 73.79; H, 5.38; N, 3.49.

4.3.6. 4-[2-(2-Fluoro-4-biphenyl)propionamido]phenyl acetate (6C). Yield 90%; mp 165–168 °C; IR (KBr): 3357, 1732, 1684, 1539, 1509, 764 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.62 (d, 3H), 2.27 (s, 3H), 3.68–3.71 (q, 1H), 7.09 (br s, 1H), 6.98–7.03 (m, 2H), 7.16–7.23 (m, 2H), 7.32–7.49 (m, 6H), 7.52–7.56 (m, 2H). Anal. Calcd for $C_{23}H_{20}FNO_3$: C, 73.20; H, 5.34; N, 3.71. Found: C, 72.77; H, 5.06; N, 3.95.

4.3.7. 4-[2-(4-Biphenyl)acetamido]phenyl acetate (7C). Yield 90%; mp 185–188 °C; IR (KBr): 3176, 1751, 1646, 1549, 1501, 746 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.26 (s, 3H), 3.77 (s, 2H), 7.15 (br s, 1H), 6.98–7.03 (m, 2H), 733–7.48 (m, 7H), 7.57–7.63 (m, 4H). Anal. Calcd for $C_{22}H_{19}NO_3$: C, 76.50; H, 5.54; N, 4.06. Found: C, 76.84; H, 5.81; N, 3.72.

4.4. Antipyretic activity

Antipyretic activity of synthesized compounds (**1B**–**7C**) was determined on rats using lipopolysaccharide (LPS) (from *E. coli*) endotoxin for producing pyrexia in rat.[17] Male Sprague–Dawley rats (150–200 g) were fasted for 16–18 h before use and were divided into parallel groups ($n = 5$). The animals were placed temporarily in restrainer and the resting rectal temperature was recorded using a flexible temperature probe connected to Bio-Pac data acquisition system. The same probe and system were used for all animals to reduce experimental error. The animals were returned to their respective cages after the temperature measurement. Animals were injected either normal saline or LPS (0.36 mg/kg, Sigma, USA) intraperitoneally and the rectal temperature was measured at 0, 5, 6 and 7 h after LPS injection. At 5 h when the increase in rectal temperature had reached a plateau, the LPS injected rats were given orally either vehicle (1% CMC) or test compound (suspended in 1% CMC) to determine whether the rise in temperature could be reversed. Percent reversal (Antipyretic activity) was calculated using the rectal temperature obtained at 7 h taking this value in the vehicle control group as zero reversal.

4.5. In vitro COX inhibition assay

COX-2 inhibiting activity for the synthesized compound and parent drugs was determined[18] using the colorimetric ovine cyclooxygenase (COX) assay kit (Cayman Chemical Company, MI, USA). This assay analyzes the peroxidase activity of the enzymes using *N*,*N*,*N*′,*N*′-tetramethylphenylenediamine (TMPD) as the reducing co-substrate.[22] Valdecoxib was used as the positive control for COX-2 inhibition. The compounds were dissolved in DMSO and diluted in the assay buffer before use. The assays were run according to the manufacturer's instructions.

4.6. In vivo carrageenan-induced rat paw edema assay

Anti-inflammatory activity was determined by using carrageenan-induced rat paw edema method described by Winter et al.[19] Fasted male Sprague–Dawley rats (150–200 g) were divided in parallel groups ($n = 5$) and were given orally either the vehicle (1% CMC) or test compound as suspension in 1% CMC. The activity was carried out on equimolar basis to the parent drug. A line was drawn using permanent marker at the ankle of the left hind paw to define the arc of the paw to be monitored. The paw volume (V_{0h}) was measured using a Plethysmometer (Ugo-Basile, Italy). The animals were then injected subplantarly with 50 μl of 1% carrageenan (Sigma, USA) in normal saline solution (i.e., 500 μg carrageenan per paw). Three hours after the carrageenan injection, the paw volume (V_{3h}) was measured, and the increase in paw volume ($V_{3h} - V_{0h}$) was calculated. The increase in paw volume was compared with that in the vehicle control group, and percent inhibition was calculated taking the values in the control group as 0% inhibition.

4.7. Analgesic activity

Analgesic activity of the derivatives was evaluated using the acetic acid writhing model in mice as described by Koster et al.[20] Swiss albino mice (18–25) divided in

parallel groups ($n = 6$) were given orally either the vehicle (1% CMC) or test compound as suspension in 1% CMC. After 1 h of oral administration the writhing syndrome was elicited by intraperitoneal administration of acetic acid (10 ml/kg body weight, 0.7% in normal saline) and number of writhes for each mice was counted after 5 min of injection for a period of 20 min. The average number of writhes in each group of compound treated mice was compared with that of the control and degree of analgesia was expressed as percent inhibition calculated according to the formula:

$$\%\ \text{inhibition of writhing} = (1 - T/S) \times 100,$$

where S is the number of writhes in control group of mice and T is the number of writhes in compound treated group of mice.

4.8. Ulcerogenic effect[21]

Sprague–Dawley rats ($n = 6$) of either sex were fasted for 36 h with water ad libitum prior to administration of the derivative. The animals were further kept on fasting for 4 h after dosing. The derivatives as suspension in 1% CMC or vehicle (1% CMC) were administered orally. The animals were sacrificed by cervical dislocation and their stomach was dissected out, cut along the greater curvature, washed with normal saline and kept in formalin solution (5%) for 15 min and gastric mucosa was observed for the lesions using a 2×2 binocular magnifier and the ulcer index was determined using the following formula as given below:

$$\text{Ulcer index} = 10(\text{Au}/\text{Am}),$$

where $\text{Au} = A_1 + \text{Ac} + \text{Ap}$, A_1 is the area of linear lesions ($l * b$), Ac is the area of circular lesions (Πr^2), Ap is the total no. of petechiae/5, and Am is the total mucosal area ($\Pi D^2/8$) (D = diameter of stomach).

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