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Design, Synthesis and Biological Evaluation of Benzimidazole/Benzothiazole and Benzoxazole Derivatives as Cyclooxygenase Inhibitors

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Abstract—We have synthesised a series of 2-[[2-alkoxy-6-pentadecylphenyl)methyl]thio]-1H-benzimidazoles/benzothiazoles and benzoxazoles from anacardic acid and investigated their ability to inhibit human cyclooxygenase-2 enzyme (COX-2). The active compounds were screened for cyclooxygenase-1 (COX-1) inhibition. Compound **13** is 384-fold and **19** is more than 470-fold selective towards COX-2 compared to COX-1. Thus, this class of compounds may serve as excellent candidates for selective COX-2 inhibition.

Cyclooxygenase (COX) is the key enzyme which catalyses the conversion of arachidonic acid to prostaglandins and thromboxanes.[1,2] There are two types of cyclooxygenase enzymes, COX-1 and COX-2. COX-1 is a constitutive enzyme, produced in many tissues such as the kidney and the gastrointestinal tract, while COX-2 is inducible and is expressed during inflammation at a site of injury.[3–5] Prostaglandins made by COX-1 enzyme are protective prostaglandins, the presence of which leads to normal renal function in the kidneys,[6] whereas, prostaglandins made by COX-2 cause inflammation.[7] Currently available NSAIDs (Nonsteroidal anti-inflammatory drugs) inhibit both COX-1 and COX-2 enzymes.[8] Inhibition of COX-1 reduces the basal production of cytoprotective PGE_2 and PGI_2 and hence causes ulceration. Therefore complete inhibition of COX-1 is not preferred and drugs that inhibit the COX-2 enzyme are better anti-inflammatory agents.

Anacardic acid (pentadecyl salicylic acid) is a phenolic constituent present in cashew (*Anacardium occidentale* L.) nut shells and is reported to exhibit variety of biological activities.[9] It is known to inhibit medicinally important enzymes such as, prostaglandinsynthase.[10] tyrosinase[11] and 5-lipoxygenase.[12] The biological activity of anacardic acid has stimulated many researchers to derive drug analogues for different application.[13,14]

Recently, we reported a novel method for isolation of anacardic acid from cashew nut shell liquid (CNSL).[15] More recently, we reported dialkyl 1,4-dihydro(2′-alkoxy-6′-pentadecylphenyl)-2,6-dimethyl-3,5-pyridine dicarboxylates as selective T-type calcium channel blockers.[16] More over, Ringbom et al. reported long chain fatty acids are potent and selective cyclooxygenase inhibitors.[17] This discovery made us to turn our interest towards synthesis of new class of cyclooxygenase inhibitors from anacardic acid. Hence, we have designed a new series of tricyclic compounds, which belongs to group of benzimidazole, benzoxazole and benzothiazole derivatives, which are known to possess anti-inflammatory activity.[18–21] Here, we report synthesis and biological evaluation of above said compounds from anacardic acid.

Title compounds (**8**–**22**) were synthesised from anacardic acid as shown in Scheme 1. Saturated anacardic acid **1** was obtained by hydrogenation of the ene mixture of anacardic acid.[15] This was converted to dialkylated compound by reacting with dimethyl sulfate/diethyl sulfate in acetone. Dialkylated anacardic acid was reduced to corresponding alcohol by treatment with lithium aluminium hydride in tetrahydrofuran[22] and then converted to chloro compound by reacting with

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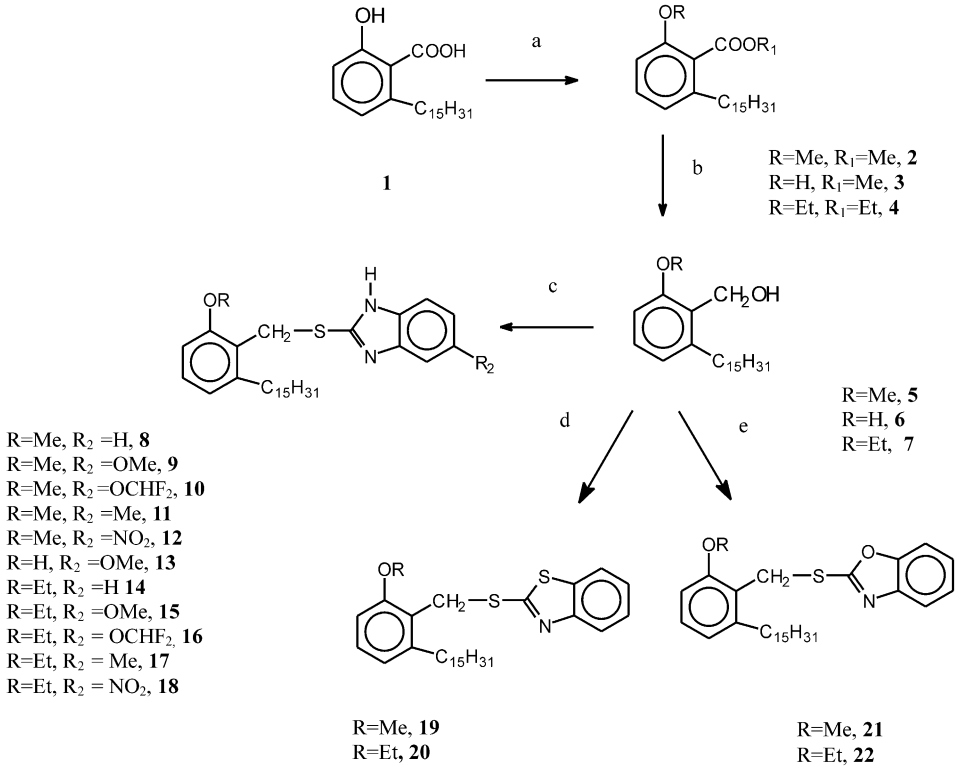


Scheme 1. Synthesis of cyclooxygenase inhibitor from anacardic acid. Reagents and conditions: (a) $(R)_sSO_4$ (R = Me/Et), K_2CO_3, acetone; (b) $LiAlH_4$, THF; (c) $SOCl_2$, dichloromethane, 20% NaOH, $(C_4H_9)_4NBr$, substituted 2-mercapto benzimidazole; (d) $SOCl_2$, dichloromethane, 20% NaOH, $(C_4H_9)_4NBr$, 2-mercapto benzothiazole; (e) $SOCl_2$, dichloromethane, 20% NaOH, $(C_4H_9)_4NBr$, 2-mercapto benzoxazole.

thionyl chloride in dichloromethane. Resultant chloro compound was condensed with substituted 2-mercapto-benzimidazole/benzoxazole/benzothiazoles in dichloromethane solution containing 20% aqueous sodium hydroxide and tetrabutyl ammonium bromide as phase transfer catalyst.[23] Obtained compounds were recrystallised in ethanol to yield title compounds. All the compounds were characterised by IR, ^1H NMR, ^{13}C NMR and mass spectroscopy.

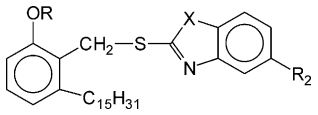
The synthesized compounds were tested for their ability to inhibit human cyclooxgenase-2 (COX-2) enzyme and the more active compounds were tested for cyclooxgenase-1 (COX-1) inhibition in human whole blood assay.[24,25] Rofecoxib was used as active control in cyclooxygenase inhibition assay.

In the case of the methoxy series (R = Me) compound **11** having a methyl group and compound **12** having nitro group on 5-position of benzimidazole ring showed moderate activity towards COX-2 inhibition. Compound **19** possessing benzothiazole in place of benzimidazole exhibited more inhibition ($IC_{50} = 1.06$ μM) when compare to **11**. In the case of **21**, which bears benzoxazole ring exhibited 2.5-fold less inhibition compared to **11**. In the case of ethoxy series, compound **14** had no substitution on benzimidazole showed 1.8-fold more inhibition with reference to **11**. Compound **15** bearing methoxy substituent on benzimidazole ring exhibited very weak inhibition ($IC_{50} = 6.25$ μM). Compound **18** having nitro group on the benzimidazole same as **12** exhibited similar inhibition. Compound **13** possessing free phenolic function on the phenyl ring and methoxy group on benzimidazole exhibited highest activity ($IC_{50} = 1$μM) among tested compounds. Remaining compounds are less active with an IC_{50} more than 10 μM.

The compound which shown IC_{50} less than 10 μM concentration were tested for COX-1 inhibition. Interestingly two compounds, namely **13** and **19** shown good activity with high selectivity towards COX-2 inhibition when compared to rest of the compounds. Compound **13** is 384 times more selective towards COX-2 when compared to COX-1 (COX-1 $IC_{50} = 384$ μM; COX-2 $IC_{50} = 1$ μM). Surprisingly compound **19** is 470 times more selective towards COX-2 inhibition than COX-1 (COX-1 $IC_{50} = > 500$; COX-2 $IC_{50} = 1.06$ μM), interestingly 100 fold more selective than **13**. However they are more selective and less potent than rofecoxib in human whole blood assay. Although compounds **11**, **12**, **14**, **15** and **21** possess good selectivity, they have shown moderate activity towards COX-2. In conclusion, these classes of compounds may serve as excellent candidates for selective COX-2 inhibition (Table 1).

Preparation of 5-(methoxy)-2-[(2-hydrox-6-pentadecyl-phenyl)-methyl]-thio]-1H-benzimidazole 13. 2-Hydroxy-6-pentadecylbenzyl alcohol **6** was prepared by the modified procedure.[22] To a solution of compound **6** (1.0 g, 2.9 mmol) in dichloromethane (25 mL) thionyl chloride (0.51 g, 4.3 mmol) was added slowly at 15–20 °C under stirring. After the addition was complete, the solution was heated to 30–35 °C for 2 h, cooled to 10 °C and distilled water (0.5 mL) was added to decompose excess

Table 1. Inhibitory effect on COX-2 and COX-1 activity in human whole blood assay



Compd	R	R_2	X	COX-2[a] IC_{50} μM	COX-1[b] IC_{50} μM	COX-1/COX-2
8	CH_3	H	NH	>10	nt*	—
9	CH_3	OCH_3	NH	>10	nt*	—
10	CH_3	$OCHF_2$	NH	>10	nt*	—
11	CH_3	CH_3	NH	2.63	>500	>190
12	CH_3	NO_2	NH	2.27	>500	>220
13	H	OCH_3	NH	1	384	384
14	CH_2CH_3	H	NH	1.47	>500	>340
15	CH_2CH_3	OCH_3	NH	6.25	>500	>80
16	CH_2CH_3	$OCHF_2$	NH	>10	nt*	—
17	CH_2CH_3	CH_3	NH	>10	nt*	—
18	CH_2CH_3	NO_2	NH	3.84	>500	>130
19	CH_3	H	S	1.06	>500	>470
20	CH_2CH_3	H	S	>10	nt*	—
21	CH_3	H	O	2.77	>500	>220
22	CH_3CH_2	H	O	>10	nt*	
Rofecoxib	—	—	—	0.057	11.4	200

[a]COX-2 activity was evaluated in human whole blood as LPS induced PGE_2 generation.
[b]COX-1 activity was measured in Human whole blood as TXB_2 generation. IC_{50} values were estimated from dose–response curve analysed by nonlinear regression using GraphPad software and values are average of three determinations, nt* samples those have IC_{50} >10 μM for COX-2 inhibition are not tested for COX-1 inhibition.

thionyl chloride. To the resultant chloro compound, 2-mercapto-5-methoxybenzimidazole (0.59 g, 3.2 mmol), and tetrabutyl ammonium bromide (0.1 g, 0.31 mmol) were added. The pH of the solution was adjusted to 10.5 using 20% sodium hydroxide solution. The reaction mixture was stirred at room temperature for 5 h. The dichloromethane layer was separated, and washed with distilled water. The organic layer was dried over anhydrous sodium sulfate, concentrated under reduced pressure to yield a residue of 2.5 g. This product was purified on silica gel column by eluting with mixture of hexane/ethylacetate (9:1) to yield the title compound, which was further recrystallised in ethyl alcohol to give colourless solid (0.50 g, 32%), mp: 122–123 °C; IR (KBr): 3300, 2910, 2850, 2798, 1584, 1450, 1402, 1359, 1262, 1065, 988, 745 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 0.84–0.88 (3H, t, CH_3, J = 6.4 Hz), 1.24 (24H, bs, $(CH_2)_{12}$), 1.61 (2H, m, CH_2), 2.65–2.75 (2H, t, Ar CH_2, J = 8.2 Hz), 3.80 (3H, s, OMe), 4.65 (2H, s, CH_2S), 6.60–6.8 (3H, m, ArH), 6.9 (1H, s, ArH), 7.1 (1H, t, ArH).

Preparation of 2-[(2-methoxy-6-pentadecylphenyl)-methyl]-thio]-benzothiazole 19. 2-Methoxy-6-pentadecyl benzyl alcohol **5** was prepared by modified procedure.[22] The title compound was synthesised by the reaction of **5** (3.0 g, 8.6 mmol) with thionyl chloride (1.53 g, 12.9 mmol) followed by condensation with 2-mercapto-benzothiazole (1.58 g, 9.4 mmol) by the similar procedure described for **13**. Purified product was recrystallised in ethyl alcohol to give colourless solid (1.5 g, 35%), mp 42 °C; IR (KBr): 2924, 2888, 1456, 1428, 1257, 1066, 995, 751 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 0.84–0.9 (3H, t, CH_3, J = 6.7 Hz), 1.24 (24H, bs, $(CH2)_{12}$, 1.60 (2H, qt, CH_2), 2.69–2.77 (2H, t, $ArCH_2$, J = 8.17 Hz), 3.83 (3H, s, OMe), 4.76 (2H, s, CH_2S), 6.72–6.76 (1H, d, ArH, J = 8.19 Hz), 6.79 (1H, d, ArH, J = 7.65 Hz), 7.3 (3H, m, ArH), 7.73 (1H, d, J = 7.9 Hz), 7.88 (1H, d, ArH, J = 7.4); mass: 497 (M^+), 464, 331, 280, 180, 161, 135 (BP), 108, 105, 91, 69, 55; Anal: C 72.38%, H 8.70%, N 2.81%, calcd for $C_{30}H_{43}NOS_2$, C 72.76%, H 8.75%, N 2.78%.

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