European Journal of Medicinal Chemistry 45 (2010) 3162–3168

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological screening of some novel amidocarbamate derivatives of ketoprofen

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ARTICLE INFO

Article history:
Received 15 September 2009
Received in revised form
30 March 2010
Accepted 7 April 2010
Available online 14 April 2010

Keywords:
Ketoprofen
Amidocarbamate
Antioxidant
Peroxidation
Lipoxygenase
Antiviral

ABSTRACT

A series of novel ketoprofen derivatives **4a**–**j** bearing both amide and carbamate functionalities were prepared using benzotriazole. Selective reduction of ketoprofen produced hydroxy derivative **2**, which reacts with one or 2 mol of 1-benzotriazole carboxylic acid chloride (**1**) gave benzotriazole derivatives **3a** and **3b** respectively. Antioxidative screenings revealed that the prepared compounds **3b** and **4a**–**j** possess excellent lipid peroxidation inhibition at 0.1 mM concentration. Two of the compounds **3b** and **4g** also showed high soybean lipoxygenase inhibition activity, where as the amidocarbamate derivatives of ketoprofen showed only weak reducing activity against 1,1-diphenyl-2-picrylhydrazyl radicals. No selective antiviral effects were noted for the tested compounds against a broad variety of DNA and RNA viruses.

1. Introduction

Ketoprofen (Ket) is a non-steroidal anti-inflammatory drug (NSAID) with pronounced analgesic and antipyretic properties. Various ketoprofen derivatives have been synthesized in order to minimize side-effects, prolong plasma half-life and increase solubility [1,2]. Some amides have proved to be useful prodrugs, while the others possess anti-inflammatory activity independent of the parent compound. It has been demonstrated that amidation of NSAIDs improves selectivity towards COX-2 [3], while modification of the carboxylic group to hydroxamic acid leads to inhibition of both cyclooxygenase and 5-lipoxygenase, these two enzymes are crucial in inflammatory processes [4,5]. Glycine amides of ketoprofen and several other well-known NSAIDs are significantly less irritating to gastric mucosa, while their anti-inflammatory activities are comparable to their parent drugs [6]. Numerous studies suggest that NSAIDs are promising anticancer drugs and may be associated with reduced risk of colon, lung, liver and other types of cancers [7,8]. In this paper, a series of novel amidocarbamate derivatives of ketoprofen **4a**–**j** was prepared, characterized and screened for their antioxidative, cytostatic and antiviral activities.

2. Chemistry

Benzotriazolides **3a**, **3b** were prepared from the reduced ketoprofen derivative **2** and 1-benzotriazole carboxylic acid chloride (**1**), presented in Scheme 1 [9,10]. If the reaction was performed with 1 equivalent of chloride **1** then product **3a** with free hydroxy group was obtained in 75% yield. When the reaction was carried out with 2 equivalents of chloride **1**, the main product was benzotriazolide **3b**, in which both carboxylic and hydroxy groups were acylated. Minor amount of product **3a** was detected, even if the reaction was performed with the excess of chloride **1**. The reaction was carried out at room temperature in order to avoid benzotriazolide **3a** polycondensation. Compounds **4a**–**j** was prepared by the reaction of benzotriazolide **3b** with 2 equivalents of an appropriate amine, in the presence of 5 equivalents of triethylamine (Scheme 1). All reactions were performed in toluene, at room temperature, for 0.5–48 h. Triethylamine formed a water soluble salt with benzotriazole, a by-product of the reaction, which was readily extracted with water. Structures of compounds **3a**, **3b** and **4a**–**j** were deduced from the analysis of their IR, ^{1}H and ^{13}C NMR spectra and confirmed by the elemental analysis. The chemical shifts were consistent with the proposed structures of the novel compounds. The physicochemical data of the newly synthesized compounds were presented in Table 1.

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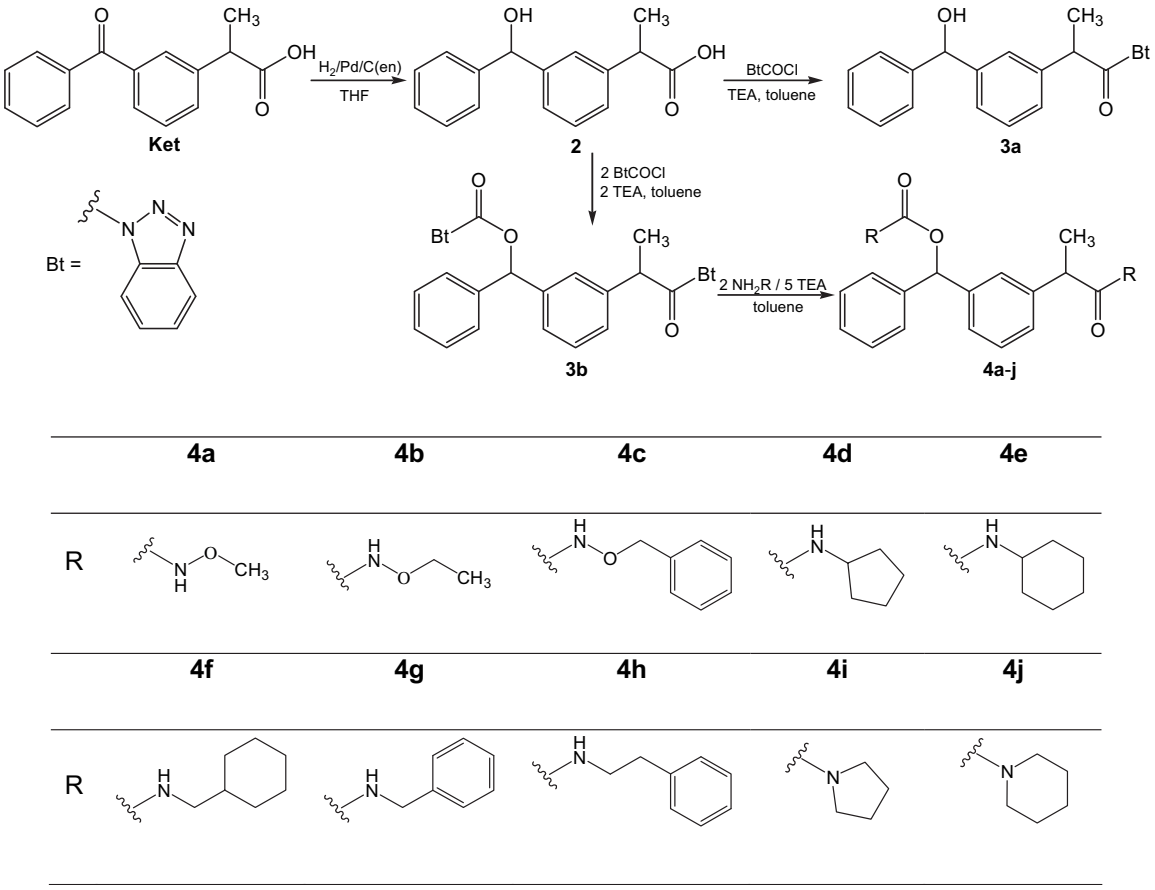
doi:10.1016/j.ejmech.2010.04.008



Scheme 1. Synthesis of compounds **4a**–**j**.

3. Pharmacological results and discussion

3.1. Biological studies

3.1.1. Antioxidant activity

The interaction of the examined compounds with the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was studied. Interaction with DPPH indicates radical scavenging ability in an iron-free system. Interactions were monitored after 20 and 60 min at two concentrations of DPPH (0.05 and 0.1 mM). Ketoprofen, the prototype compound, benzotriazolide **3b** as well as all the tested compounds presented very low interaction values. The results are presented in Table 2.

3.1.2. Soybean lipoxygenase inhibition

Compounds were further evaluated for the inhibition of soybean lipoxygenase (LOX) by the UV absorbance based enzyme assay [11]. Lipoxygenases oxidize certain fatty acids at specific positions to hydroperoxides, precursors of leukotrienes, which contain a conjugated triene structure, i.e. soybean lipoxygenase converts linoleic to 13-hydroperoxylinoleic acid. Leukotrienes play an important role as inflammatory and allergic mediators [12]. Inhibitors of LOX have attracted attention initially as potential agents for the treatment of inflammatory and allergic diseases but their therapeutic potential has now been expanded to certain types of cancer and cardiovascular diseases [13]. Most of the LOX inhibitors are antioxidants or free radical scavengers, since lipoxygenation occurs via a carbon-centered radical [14]. According to IC_{50} values compound **3b** is the most active as compared to compounds **4g**, **4d** and **4f** (IC_{50} = 21–95 μM). From Table 2 it is obvious that aromatic and cycloalkyl derivatives **4g**, **4d** and **4f** are more potent lipoxygenase inhibitors than the other amidocarbamates.

3.1.3. Inhibition of linoleic acid lipid peroxidation

Azo compounds generating free radicals through spontaneous thermal decomposition are useful for free radical production studies *in vitro*. The water soluble azo compound 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) has been extensively used as a clean and controllable source of thermally produced alkylperoxyl free radicals. In this study AAPH was used as a free radical initiator to follow oxidative changes of linoleic acid to conjugated diene hydroperoxide. The results indicated that all the compounds are excellent inhibitors of lipid peroxidation (LP) (54.5–99.5%), significantly higher than ketoprofen (69.3%) at 0.1 mM concentration (Table 2). This inhibition was found to be concentration dependent.

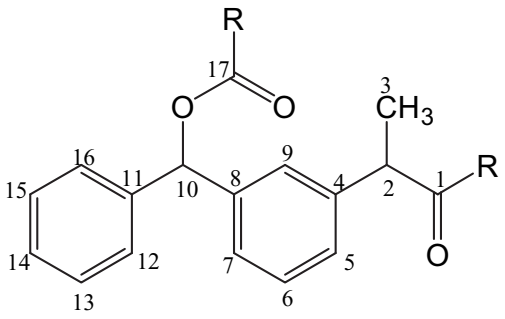
Regression analysis of the lipid peroxidation inhibition at 100 μM revealed that the overall molar refractivity (CMR) is the main physicochemical parameter influencing the inhibition. The linear CMR model suggests that the compounds with high CMR value will be more active. No correlation for lipophilicity was found.

$$\log LP\% = 0.047(0.018)CMR - 1.317(0.234)$$
$$n = 11, r = 0.894, r^2 = 0.800, q^2 = 0.680,$$
$$s = 0.045, F_{1,8} = 31.555, a = 0.01$$

3.1.4. Antiviral and cytostatic evaluation

Antiviral evaluation was performed on a series of DNA and RNA viruses: herpes simplex virus type 1 (HSV-1), HSV-2 (G), vaccinia

Table 1
Physicochemical properties of compounds **4a**–**j**.



4a - 4j

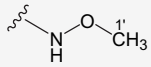
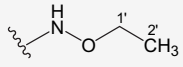
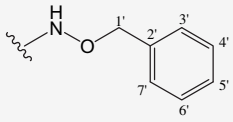
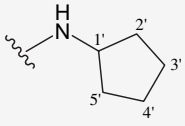
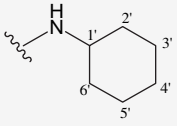
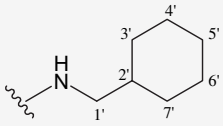
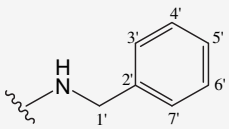
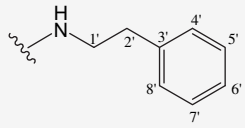
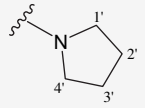
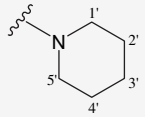
Compd. No.	R	Molecular formula	Molecular weight	Elemental analysis data			^{1}H and ^{13}C NMR (DMSO-d_6, δ/ppm, J/Hz)
				C	H	N	
4a		$C_{19}H_{22}N_2O_5$	358.34	63.64	6.18	7.82	11.22 (s, 1H, NH carbamate), 10.65 (s, 1H, NH amide), 7.39–7.21 (m, 9H, arom.), 6.71 (s, 1H, 10), 3.58 and 3.51 (2s, 3H, 1′),[a] 3.38 (q, 1H, 2, J = 6.79), 1.31 (d, 3H, 3, J = 7.02) 170.31 (17), 156.20 (1), 142.14, 141.10, 140.98 (4, 8, 11), 128.97, 128.27, 127.21, 126.96, 125.68, 125.42 (5–7, 9, 12–16), 77.15 (10), 64.03 and 63.49 (1′),[a] 42.49 (2), 18.63 (3)
4b		$C_{21}H_{26}N_2O_5$	386.28	65.22	6.77	7.24	11.09 (s, 1H, NH carbamate), 10.54 (s, 1H, NH amide), 7.36–7.21 (m, 9H, arom.), 6.69 (s, 1H, 10), 3.81–3.66 (m, 3H, 2, 1′) and 3.42–3.33 (m, 2H, 1′),[a] 1.30 (d, 3H, 3, J = 6.99), 1.15–1.04 (m, 6H, 2′) 170.37 (17), 156.30 (1), 142.25, 141.13, 141.07 (4, 8, 11), 128.95, 128.23, 127.18, 126.94, 125.62, 125.37 (6–8, 10, 13–17), 77.12 (10), 71.50 and 70.87 (1′),[a] 42.48 (2), 18.61 (3), 13.87, 13.83 (2′)[a]
4c		$C_{31}H_{30}N_2O_5$	510.64	72.91	5.92	5.42	11.21 (s, 1H, NH carbamate), 10.66 (s, 1H, NH amide), 7.38–7.21 (m, 19H, arom.), 6.72 (s, 1H, 10), 4.77 and 4.70 (2s, 4H, 1′),[a] 3.41 (q, 1H, 3, J = 6.96), 1.31 (d, 3H, 3, J = 6.86) 170.54 (17), 156.43 (1), 142.16, 141.08, 141.06 (4, 8, 11), 136.35, 136.27 (2′), 129.37, 129.26, 128.74, 128.71 (3′–7′), 128.96, 128.25, 127.32, 126.96, 125.74, 125.42 (5–7, 9, 12–16), 77.92 and 77.09 (1′),[a] 77.33 (10), 42.44 (2), 18.68 (3)
4d		$C_{27}H_{34}N_2O_3$	434.44	74.62	7.88	6.44	7.87 (d, 1H, NH carbamate, J = 6.90), 7.44 (d, 1H, NH amide, J = 6.90), 7.34–7.17 (m, 9H, arom.), 6.63 (s, 1H, 10), 3.98–3.87 and 3.81–3.73 (2m, 1H, 1′),[a] 3.55 (q, 1H, 2, J = 6.96), 1.83–1.16 (m, 16H, 2′–5′), 1.28 (d, 3H, 3, J = 7.00) 172.86 (17), 155.26 (1), 143.12, 141.80, 141.65 (4, 8, 11), 128.81, 128.66, 127.94, 127.01, 126.91, 125.87, 125.09 (5–7, 9, 12–16), 76.39 (10), 52.61 and 50.65 (1′),[a] 45.18 (2), 32.78 and 32.61 (2′)[a] 32.69 (5′), 23.95 and 23.91 (3′),[a] 23.73 (4′), 18.99 (3)
4e		$C_{29}H_{38}N_2O_3$	462.54	75.26	8.27	6.05	7.75 (d, 1H, NH carbamate, J = 7.77), 7.36–7.17 (m, 10H, arom., NH amide), 6.62 (s, 1H, 10), 3.55 (q, 1H, 2, J = 6.94), 3.50–3.40 and 3.29–3.17 (2m, 1H, 1′),[a] 1.74–1.52, 1.28–0.96 (2m, 20H, 2′–6′), 1.27 (d, 3H, 3, J = 7.01) 172.42 (17), 154.96 (1), 143.16, 141.80, 141.66 (4, 8, 11), 128.78, 128.63, 127.91, 127.01, 126.92, 125.81, 125.10 (5–7, 9, 12–16), 76.37 (10), 50.01 and 47.85 (1′),[a] 45.23 (2), 33.11 (2′), 32.78 (6′), 25.68 and 25.61 (3′),[a] 25.06 (4′) 24.97 (5′), 18.95 (3)
4f		$C_{31}H_{42}N_2O_3$	490.54	75.85	8.64	5.68	7.86 (t, 1H, NH carbamate, J = 5.48), 7.44–7.16 (m, 10H, arom., NH amide), 6.61 (s, 1H, 10), 3.59 (q, 1H, 2, J = 6.88), 2.87–2.80 (m, 4H, 1′), 1.72–1.53 (m, 10H, 2′, 3′, 7′), 1.41–1.28, 1.20–1.04, 0.88–0.72 (3m, 12H, 4′–6′), 1.29 (d, 3H, 3, J = 7.00) 173.42 (17), 155.93 (1), 143.10, 141.78, 141.59 (4, 8, 11), 128.80, 128.62, 127.96, 127.02, 125.85, 125.10 (5–7, 9, 12–16), 76.48 (10), 47.07 and 45.25 (1′),[a] 45.45 (2), 38.13 and 37.86 (2′),[a] 30.80 and 30.77 (3′),[a] 30.71 (7′), 26.49, 25.83 (4′–6′), 19.02 (3)
4g		$C_{31}H_{30}N_2O_3$	478.52	77.78	6.31	5.85	8.49–8.44 (dd, 1H, NH carbamate, J = 3.71, J = 5.25), 8.04–8.00 (m, 1H, NH amide), 7.38–7.13 (m, 19H, arom.), 6.67 (s, 1H, 10), 4.24–4.19 (m, 4H, 1′), 3.66 (q, 1H, 2, J = 6.83), 1.34 (d, 3H, 3, J = 6.90) 173.54 (17), 156.10 (1), 142.92, 141.68, 141.53 (4, 8, 11), 140.07, 139.92 (2′), 128.88, 128.77, 128.05, 127.25, 126.99, 126.09, 125.26 (5–7, 9, 12–16), 128.73, 128.69, 127.51, 127.40, 127.15 (3′–7′), 76.82 (10), 45.51 (2), 44.27 and 42.51 (1′),[a] 19.07 (3)

Table 1 (*continued*)

Compd. No.	R	Molecular formula	Molecular weight	Elemental analysis data			^{1}H and ^{13}C NMR (DMSO-d_6, δ/ppm, J/Hz)
				C	H	N	
4h		$C_{33}H_{34}N_2O_3$	506.48	78.20	6.76	5.53	8.01 (t, 1H, NH carbamate, J = 5.05), 7.54 (t, 1H, NH amide, J = 5.48), 7.34–7.08 (m, 19H, arom.), 6.64 (s, 1H, 10), 3.55 (q, 1H, 2, J = 6.88), 3.28–3.20 (m, 4H, 1′), 2.66–2.50 (m, 4H, 2′), 1.28 (d, 3H, 3, J = 7.16) 173.40 (17), 155.73 (1), 142.94, 141.71, 141.54 (4, 8, 11), 139.87, 139.70 (3′), 128.83, 127.98, 127.12, 127.00, 125.97, 125.19 (5–7, 9, 12–16), 129.10, 128.75, 128.68, 126.53, 126.44 (4′–8′), 76.54 (10), 45.47 (2), 42.39 and 40.73 (1′),[a] 35.79 and 35.49 (2′),[a] 19.10 (3)
4i		$C_{25}H_{30}N_2O_3$	406.46	73.83	7.44	6.88	7.39–7.17 (m, 9H, arom.) 6.67 (s, 1H, 10), 3.87 (q, 1H, 2, J = 6.62), 3.57–3.46, 3.30–3.14 and 3.03–2.95 (3m, 1′, 4′),[a] 1.89–1.62 (m, 8H, 2′, 3′), 1.27 (d, 3H, 3, J = 6.77) 171.51 (17), 153,46 (1), 142.55, 142.04, 141.81 (4, 8, 11), 129.15, 128.91, 127.99, 127.04, 126.79, 125.94, 125.10, (5–7, 9, 12–16), 76.85 (10), 46.45 and 46.18 (1′),[a] 46.07 and 46.00 (4′),[a] 43.71 (2), 25.98 and 25.72 (2′),[a] 24.90 and 24.16 (3′)[a], 20.31 (3)
4j		$C_{27}H_{34}N_2O_3$	434.53	74.58	7.85	6.42	7.34–7.17 (m, 9H, arom.), 6.66 (d, 1H, 10, J = 2.07), 4.05 (q, 1H, 2, J = 6,65), 3.74–3.70, 3.59–3.43 and 3.21–3.01 (3m, 8H, 1′, 5′),[a] 1.59–1.31, 1.18–1.07, 0.62–0.48 (3m, 12H, 2′–4′), 1.24 (dd, 3H, 3, J = 3.15, J = 3.59) 171.04 (17), 153,87 (1), 143.37, 142.06, 141.64 (4, 8, 11), 129.25, 128.90, 127.99, 127.08, 126.87, 126.66, 125.36, 125.16, 124.64 (5–7, 9, 12–16), 77.28 (10), 46.27 and 44.89 (1′),[a] 42.81 and 42.79 (5′),[a] 41.85 (2), 25.76 and 25.73 (2′),[a] 25.51 and 24.40 (3′),[a] 24.24 (4′),[a] 21.10 (3)

[a] The first signals belong to the carbamate 1′, 2′, 3′, 4′ or 5′ atoms, while the second signals belong to amide 1′, 2′, 3′, 4′ or 5′ atoms.

virus, vesicular stomatitis virus, parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4, Punta Toro virus, vesicular stomatitis virus, Coxsackie virus B4, respiratory syncytial virus, influenza A (H1N1; H3N2), influenza B, HIV-1(III_B) and HIV-2(ROD). No selective antiviral effects were noted for any of the tested compounds against any of the viruses evaluated at subtoxic compound concentrations (data not shown). Only **4g** showed minor antiviral activity against vesicular stomatitis virus, Coxsackie virus B4 and Punta Toro virus. However, the activity was found at compound concentrations close to their cytostatic activities, pointing to a toxic rather than a specific antiviral effect. The compounds have also been evaluated for their cytostatic activity against murine leukemia L1210, murine mammary carcinoma FM3A and human T-lymphoblast CEM cell cultures. The 50% inhibitory concentrations of the test compounds ranked between 2.7 μM and 422 μM depending on the nature of the compound and the tumor cell line evaluated (Table 3). The majority of the compounds show IC_{50} values around 10–25 μM (i.e. **4c**, **4d**, **4e**, **4f**, **4g**, **4h** and **4j**), pointing to a relatively minor role of the R-substituents on the core structure for cytostatic activity, as long as a bulky lipophilic (cyclic) entity has been present. Also, the presence of the amide groups might play an important role to eventually exert cytostatic potential.

4. Conclusions

A series of novel ketoprofen amidocarbamate derivatives **4a**–**j** were prepared and screened for antioxidative, antiviral and cytostatic activities. Antioxidative screenings revealed that the prepared compounds possess excellent lipid peroxidation inhibition. Compounds **3b** and **4g** also showed high soybean lipoxygenase inhibition activity. No selective antiviral effects were noted for the tested compounds against a broad variety of DNA and RNA viruses. Most compounds showed a moderate cytostatic activity.

Table 2
Interaction with DPPH, *in vitro* inhibition of soybean lipoxygenase (LOX) and lipid peroxidation (LP).

Compd.	DPPH 20 min[a] (%)	DPPH 0 min[a] (%)	DPPH 20 min[b] (%)	DPPH 60 min[b](%)	LOX Inhibition[b] (%)	LP Inhibition[c] (%)	LP Inhibition[b] (%)	clog P[d]	CMR[d]
3b	2.9	3.7	2.5	5.4	95.0[f]	15.0	98.0	6.05	14.16
4a	n.a.[e]	4.1	1.8	3.2	26.9	2.5	61.0	2.17	9.71
4b	2.5	3.7	2.3	1.1	22.3	n.a.	54.5	3.23	10.64
4c	3.4	5.6	n.a.	n.a.	40.8	49.2	99.3	5.71	14.74
4d	3.2	2.8	n.a.	2.3	69.6[f]	n.a.	95.2	4.76	12.76
4e	2.0	3.1	n.a.	n.a.	22.7	5.4	96.1	5.88	13.69
4f	1.7	3.1	1.6	5.2	56.6[f]	1.4	99.5	7.12	14.62
4g	4.2	5.3	n.a.	2.4	83.8[f]	n.a.	98.4	4.33	14.43
4h	3.6	8.5	n.a.	2.2	18.9	22.5	99.0	6.01	15.36
4i	5.7	6.5	2.6	2.7	12.7	16.4	77.2	4.70	11.84
4j	8.6	10	1.7	4.3	n.a.	25.5	97.4	5.81	12.76
Ketoprofen	6.4	3.1	8.1	7.2	n.d.[g]	n.d.	69.3	n.d.	n.d.
Caffeic acid	n.d.	n.d.	n.d.	n.d.	600	n.d.	n.d.	n.d.	n.d.
NDGA	81	83	93	97	n.d.	n.d.	n.d.	n.d.	n.d.
Trolox	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	63	n.d.	n.d.

[a] Concentrations of the tested compounds: 5×10^{-5} M.
[b] Concentrations of the tested compounds: 1×10^{-4} M.
[c] Concentrations of the tested compounds: 1×10^{-5} M.
[d] Theoretically calculated values.
[e] No activity under the reported experimental conditions.
[f] IC_{50} value was also determined: 21 (**3b**), 86 (**4d**), 95 (**4f**), 32 (**4g**), 130 (Ket) μM.
[g] Not determined.

Table 3
Cytostatic activity of the test compounds in cell cultures.

Compd.	IC_{50} (μM)[a]		
	L1210	FM3A	CEM
3b	38 ± 1	194 ± 13	67 ± 31
4a	334 ± 76	10 ± 128	422 ± 110
4b	262 ± 15	275 ± 70	313 ± 25
4c	9.7 ± 0.2	11 ± 1	14 ± 0
4d	9.8 ± 0.2	12 ± 1	12 ± 2
4e	9.6 ± 0.1	15 ± 1	15
4f	11 ± 0	n.a.[b]	n.a.
4g	10 ± 1	14 ± 1	2.7 ± 0.3
4h	8.2 ± 1.0	13 ± 1	14
4i	41 ± 3	35 ± 5	42 ± 0
4j	15 ± 2	8.0 ± 5.1	13 ± 6

[a] Compound concentration required to inhibit tumor cell proliferation by 50%.
[b] No activity under the reported experimental conditions.

5. Experimental

5.1. Chemistry

Melting points were determined on a Stuart Melting Point Apparatus SMP3 (Lennox, Ireland) and were uncorrected. IR spectra were recorded on a FTIR Perkin Elmer Paragon 500 spectrometer (SE Source, Canada). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer (Lab X, Canada), operating at 300 and 75.5 MHz for the ^{1}H and ^{13}C nuclei, respectively. Samples were measured in DMSO-d_6 solutions at 20 °C in 5-mm NMR tubes. Chemical shifts (δ) were referred to TMS. Coupling constants (J) are given in Hz. Elemental analysis was determined on CHN-LECO-932 (Choice Analytical, USA) and the elemental composition of the compounds agreed within ±0.4%. For thin-layer chromatography, precoated Merck silica gel 60 F254 and solvent system cyclohexane/ethyl acetate/methanol (3:1:0.5) were used. Spots were visualized by short-wave UV light and iodine vapor. Column chromatography was performed on Merck silica gel 0.063–0.200 mm with cyclohexane/ethyl acetate (1:2, 2:1 → 1:1, 1:1) as eluents. Benzotriazole, triphosgene, triethylamine, cyclopentylamine, cyclohexylamine, cyclohexanemethylamine, benzylamine, 2-phenylethylamine, pyrrolidine, piperidine, *O*-methylhydroxylamine hydrochloride, *O*-benzylhydroxylamine hydrochloride, ethylenediamine, 10% Pd/C, DPPH, AAPH, nordihydroguaiaretic acid (NDGA), sodium linoleate, soybean lipoxygenase, caffeic acid and trolox were purchased from Sigma–Aldrich. *O*-ethylhydroxylamine hydrochloride was obtained from E. Merck, India, CDH, s.d. Fine Chem. and Qualigens, India. Ketoprofen was obtained as a gift sample from Ranbaxy Laboratories Ltd., India. All solvents were of analytical grade and were dried prior to use.

5.1.1. 1-Benzotriazole carboxylic acid chloride (BtCOCl, **1**)

BtCOCl was prepared from benzotriazole and triphosgene [9].

5.1.2. 2-(3-(Hydroxy(phenyl)methyl)phenyl)propanoic acid (**2**)

2-(3-(Hydroxy(phenyl)methyl)phenyl)propanoic acid (**2**) was prepared by the catalytic hydrogenation of ketoprofen using H_2/Pd/C(en)/tetrahydrofurane [15].

5.1.3. Benzotriazolides ***3a, b***. General procedure

To a solution of **2** (2.561 g, 10 mmol) and triethylamine (10 or 28 mmol) in dry toluene (20 or 50 mL), a solution of chloride **1** (10 or 28 mmol) in dry toluene (20 or 50 mL) was added dropwise (15 min). The reaction mixture was stirred at room temperature for 60 min and extracted 4 times with water. The organic layer was dried over anhydrous sodium sulphate, filtrated and evaporated. Thus obtained crude products were purified by trituration with ether.

5.1.4. 2-(3-(Hydroxy(phenyl)methyl)phenyl)propanoic acid benzotriazolide (***3a***)

From the reaction of **2**, chloride **1** (1.696 g, 10 mmol), and triethylamine (1.393 mL, 10 mmol) in dry toluene (2 × 20 mL). 2.681 g (75%) of **3a** was obtained; mp 96–99 °C; IR (KBr): ν_{max} 3342, 3072, 3027, 3003, 2942, 2874, 1738, 1060, 1597, 1486, 1452, 1376, 959, 771, 751, 746, 710 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.26–8.21 (m, 2H, arom.), 7.80–7.75 (m, 1H, arom.), 7.62–7.57 (m, 1H, arom.), 7.52–7.50 (m, 1H, arom.), 7.33–7.14 (m, 8H, arom.), 5.88 (d, 1H, 11, J = 4.00 Hz), 5.65 (d, 1H, 10, J = 3.83 Hz), 5.32 (q, 1H, 2, J = 6.89 Hz), 1.63 (d, 3H, 3, J = 6.94 Hz); ^{13}C NMR (DMSO-d_6) δ 173.50 (1), 146.82, 145.88, 145.83, 140.01, 131.18 (4, 8, 12, 1′, 6′), 131.39, 129.08, 128.45, 127.14, 127.02, 126.66, 126.63, 126.58, 125.84, 120.53, 114.44 (5–7, 9, 13–17, 2′–5′), 74.49 (10), 45.12 (2), 19.07 (3). Anal. ($C_{22}H_{19}N_3O_2$) C, H, N.

5.1.5. 2-(3-(N-Benzotriazolcarbonyloxy)(phenyl)(methyl)phenyl) propanoic acid benzotriazolide (***3b***)

From the reaction of **2**, chloride **1** (5.080 g, 28 mmol) and triethylamine (3.9 mL, 28 mmol) in dry toluene (2 × 50 mL) 3.52 g (70%) of **3b** was obtained; mp 124–127 °C; IR (KBr): ν_{max} 3091, 3032, 2980, 2937, 1764, 1732, 597, 1486, 1451, 1398, 1250, 1036, 951, 781, 760, 748, 708, 583 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.28–7.98 (m, 3H, arom.), 7.78–7.28 (m, 15H, arom.), 7.24 (s, 1H, 10), 5.38 (q, 1H, 2, J = 6.84 Hz), 1.66 (d, 3H, 3, J = 6.94 Hz); ^{13}C NMR (DMSO-d_6) δ 173.28 (1), 147.91 (1″), 145.80, 145.77, 140.72, 140.02, 139.17, 131.73 (4, 8, 11, 1′, 6′, 2″, 7″), 131.36, 131.11, 129.83, 129.20, 128.29, 127.36, 127.23, 126.97, 126.79, 126.55, 126.38, 120.70, 120.45, 114.39, 113.67 (5–7, 9, 12–16, 2′–5′, 3″–6″), 81.48 (10), 45.04 (2), 18.98 (3). Anal. ($C_{29}H_{22}N_6O_3$) C, H, N.

5.1.6. Amidocarbamates ***4a–j***. General procedure

A solution of benzotriazolide **3b** (0.5 mmol), the appropriate amine (1.1 mmol) and triethylamine (0.348 mL, 2.5 mmol) in toluene (5 mL) was stirred at room temperature for 30 min–48 h. The reaction mixture was extracted with brine (5 × 10 mL), hydrochloric acid (w = 1%, 1 × 5 mL) and washed with water till pH 7. After drying (anhydrous sodium sulphate) and evaporation of the solvent, the crude product was obtained.

5.1.7. (3-(1-(Methoxycarbamoil)ethyl)phenyl)(phenyl)methyl methoxycarbamate (***4a***)

To the product **3b**, *O*-methylhydroxylamine hydrochloride (0.092 g, 0.5 mmol) and triethylamine was added at room temperature and stirred for 10 h. After purification by the column chromatography (eluent cyclohexane/ethyl acetate 1:2) 0.143 g (80%) of **4a** was obtained; oil; IR (film): ν_{max} 3455, 3217, 3065, 2978, 2938, 1725, 1668, 1606, 1489, 1454, 1254, 1115, 1043, 705 cm^{-1}. Anal. ($C_{19}H_{22}N_2O_5$) C, H, N.

5.1.8. (3-(1-(Ethoxycarbamoil)ethyl)phenyl)(phenyl)methyl ethoxycarbamate (***4b***)

To the product **3b**, *O*-ethylhydroxylamine hydrochloride (0.107 g, 0.5 mmol) and triethylamine was added at room temperature and stirred for 25 h. After purification by the column chromatography (eluent cyclohexane/ethyl acetate 2:1 → 1:1) 0.140 g (73%) of **4b** was obtained; oil; IR (film): ν_{max} 3454, 3219, 3064, 2981, 2937, 2891, 1724, 1715, 1668, 1606, 1494, 1454, 1384, 1253, 1113, 1041, 704 cm^{-1}. Anal. ($C_{21}H_{26}N_2O_5$) C, H, N.

5.1.9. (3-(1-(Benzyloxycarbamoil)ethyl)phenyl)(phenyl)methyl benzyloxycarbamate (***4c***)

To the product **3b**, *O*-benzylhydroxylamine hydrochloride (0.175 g, 0.5 mmol) and triethylamine was added at room temperature and stirred for 48 h. After purification by the column chromatography (eluent cyclohexane/ethyl acetate 1:1) 0.115 g

(45%) of **4c** was obtained; mp 42–45 °C; IR (KBr): ν_{max} 3215, 3064, 3032, 2974, 2936, 1722, 1665, 1605, 1495, 1454, 1249, 1107, 1027, 749, 699 cm^{-1}. Anal. ($C_{31}H_{30}N_2O_5$) C, H, N.

*5.1.10. (3-(1-(Cyclopentylcarbamoil)ethyl)phenyl)(phenyl)methyl cyclopentylcarbamate (**4d**)*

To the product **3b**, cyclopentylamine (0.109 mL, 0.5 mmol) and triethylamine was added at room temperature and stirred for 1 h. After trituration with ether 0.167 g (77%) of **4d** was obtained; mp 132–135 °C; IR (KBr): ν_{max} 3305, 3269, 3065, 2962, 2870, 1700, 1651, 1606, 1545, 1452, 1249, 1040, 1017, 702 cm^{-1}. Anal. ($C_{27}H_{34}N_2O_3$) C, H, N.

*5.1.11. (3-(1-(Cyclohexylcarbamoil)ethyl)phenyl)(phenyl)methyl cyclohexylcarbamate (**4e**)*

To the product **3b**, cyclopentylamine (0.126 mL, 0.5 mmol) and triethylamine was added at room temperature and stirred for 5 h. After trituration with ether 0.173 g (75%) of **4e** was obtained; mp 139–142 °C; IR (KBr): ν_{max} 3303, 3265, 3066, 2933, 2854, 1695, 1650, 1603, 1547, 1450, 1235, 1042, 702 cm^{-1}. Anal. ($C_{29}H_{38}N_2O_3$) C, H, N.

*5.1.12. (3-(1-(Cyclohexanemethylcarbamoil)ethyl)phenyl)(phenyl) methyl cyclohexanemethylcarbamate (**4f**)*

To the product **3b**, cyclohexanemethylamine (0.143 mL, 0.5 mmol) and triethylamine was added at room temperature and stirred for 30 min. After trituration with ether 0.184 g (75%) of **4f** was obtained; mp 128–129 °C; IR (KBr): ν_{max} 3340, 3278, 3064, 2922, 2851, 1707, 1654, 1604, 1551, 1449, 1249, 702 cm^{-1}. Anal. ($C_{31}H_{42}N_2O_3$) C, H, N.

*5.1.13. (3-(1-(Benzylcarbamoil)ethyl)phenyl)(phenyl)methyl benzylcarbamate (**4g**)*

To the product **3b**, benzylamine (0.120 mL, 0.5 mmol) and triethylamine was added at room temperature and stirred for 30 min. After trituration with ether 0.127 g (53%) of **4 g** was obtained; mp 107–109 °C; IR (KBr): ν_{max} 3316, 3267, 3087, 3063, 3032, 2932, 1684, 1641, 1606, 1551, 1519, 1454, 1248, 699 cm^{-1}. Anal. ($C_{31}H_{30}N_2O_3$) C, H, N.

*5.1.14. (3-(1-(Phenylethylcarbamoil)ethyl)phenyl)(phenyl)methyl phenylethylcarbamate (**4h**)*

To the product **3b**, phenylethylamine (0.139 mL, 0.5 mmol) and triethylamine was added at room temperature and stirred for 35 min. After trituration with ether 0.190 g (75%) of **4h** was obtained; oil; IR (KBr): ν_{max} 3417, 3313, 3063, 3028, 2972, 2932, 2872, 1713, 1699, 1660, 1650, 1604, 1538, 1517, 1496, 1454, 1248, 1030, 749, 700 cm^{-1}. Anal. ($C_{33}H_{34}N_2O_3$) C, H, N.

*5.1.15. (3-(1-Oxo-1-(pyrrolidin-1-yl)propan-2-yl)phenyl)(phenyl) methyl pyrrolidine-1-carboxylate (**4i**)*

To the product **3b**, pyrrolidine (0.092 mL, 0.5 mmol) and triethylamine was added at room temperature and stirred for 30 min. After evaporation 0.152 g (75%) of **4i** was obtained; oil; IR (film): ν_{max} 3061, 3030, 2973, 2875, 1700, 1643, 1634, 1588, 1454, 1416, 1126, 1096, 764, 709 cm^{-1}. Anal. ($C_{25}H_{30}N_2O_3$) C, H, N.

*5.1.16. (3-(1-Oxo-1-(piperidin-1-yl)propan-2-yl)phenyl)(phenyl) methyl piperidine-1-carboxylate (**4j**)*

To the product **3b**, piperidine (0.109 mL, 0.5 mmol) and triethylamine was added at room temperature and stirred for 45 min. After trituration with ether 0.179 g (80%) of **4j** was obtained; mp 106–109 °C; IR (KBr): ν_{max} 3050, 3028, 2971, 2940, 2852, 1694, 1628, 1587, 1469, 1426, 1258, 1236, 1148, 1083, 1026, 707, 699 cm^{-1}. Anal. ($C_{27}H_{34}N_2O_3$) C, H, N.

5.1.17. Interaction with DPPH

To a solution of DPPH (0.05 mM) in absolute ethanol an equal volume of 0.1 or 0.05 mM ethanolic solution of the tested compound was added [11]. After 20 and 60 min the absorbance was recorded at 517 nm and compared to the appropriate standard NDGA (Table 2). Ethanol was used as a control. Each *in vitro* experiment was performed at least in triplicate and the standard deviation of absorbance was less than 10% of the mean.

5.1.18. Soybean lipoxygenase inhibition

DMSO solution of the tested compound was incubated with sodium linoleate (0.1 mM) and 0.2 mL of soybean lipoxygenase solution ($1/9 \times 10^{-4}$ *w*/*v* in saline) at room temperature [11]. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid was recorded at 234 nm and compared to the standard inhibitor caffeic acid.

5.1.19. Inhibition of linoleic acid lipid peroxidation

Oxidation of linoleic acid to conjugated diene hydroperoxide in an aqueous dispersion is monitored at 234 nm [16]. AAPH was used as a free radical initiator. Ten microliters of the 16 mM linoleic acid dispersion was added to the UV cuvette containing 0.93 mL of 0.05 M phosphate buffer, pH 7.4 prethermostated at 37 °C. The oxidation reaction was initiated at 37 °C under air by the addition of 50 μL of 40 mM AAPH solution. Oxidation was carried out in the presence of compounds (10 μL, final concentration 0.1 mM). In the assay with no antioxidant lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation was monitored at 37 °C by recording the increase of absorption at 234 nm caused by conjugated diene hydroperoxides. The results were compared to the standard inhibitor trolox.

5.1.20. Antiviral and cytostatic activity assays

Murine leukemia L1210, murine mammary carcinoma FM3A and human T-lymphocyte CEM cells were suspended at 300,000–500,000 cells/mL of culture medium, and 100 μL of a cell suspension was added to 100 μL of an appropriate dilution of the test compounds in wells of 96-well microtiter plates. After incubation at 37 °C for two (L1210, FM3A) or three (CEM) days, the cell number was determined using a Coulter counter. The IC_{50} was defined as the compound concentration required to inhibit cell proliferation by 50%.

The antiviral assays, other than the anti-HIV assays, were based on inhibition of virus-induced cytopathicity in HEL [herpes simplex virus type 1 (HSV-1), HSV-2 (G), vaccinia virus and vesicular stomatitis virus], Vero (parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4, and Punta Toro virus), HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus) or MDCK (influenza A (H1N1; H3N2); and influenza B) cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus ($CCID_{50}$ being the virus dose to infect 50% of the cell cultures). After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (200, 40, 8 μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. The methodology of the anti-HIV assays was as follows: human CEM ($\sim 3 \times 10^5$ cells/mL) cells were infected with 100 $CCID_{50}$ of HIV-1(III_B) or HIV-2(ROD)/mL and seeded in 200 μL wells of a microtiter plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, HIV-induced CEM giant cell formation was examined microscopically.

Acknowledgements

The authors are thankful to the Management and Principal for providing the research facilities to pursue this work.

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