Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic Chemistry 33 (2005) 67–81
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

Inhibitory activity of polyhydroxycarboxylate chelators against recombinant NF-κB p50 protein–DNA binding

Rakesh K. Sharma [a], Vineet Pande [b], Maria J. Ramos [b], Hament K. Rajor [a], Shilpa Chopra [a], Kazuyuki Meguro [c], Jun-ichiro Inoue [c], Masami Otsuka [d,*]

[a] *Department of Chemistry, University of Delhi, Delhi 7, India*
[b] *CEQUP/Departamento de Química, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre 687, 4169-007 Porto, Portugal*
[c] *Institute of Medical Science, University of Tokyo, Shirokane-dai, Minato-ku, Tokyo 108-0071, Japan*
[d] *Faculty of Medical and Pharmaceutical Sciences, Kumamoto University, 5-1 Oehonmachi, Kumamoto 862-0973, Japan*

Received 15 June 2004

Abstract

The inhibitory effect of 7,8-dihydroxy-4-methylcoumarin (7,8-DHMC), 5,7-dihydroxy-4-methylcoumarin (5,7-DHMC), and gallic acid on the DNA binding of recombinant p50 protein and their interaction with zinc ion were studied. Electrophoretic mobility shift assay (EMSA) using p50 and biotin labeled DNA has shown that gallic acid is more effective than the dihydroxycoumarins in inhibiting the p50–DNA binding. Molecular modeling studies suggest an explanation for these observations. Effect of the addition of zinc after p50–DNA-binding inhibition by gallic acid was also studied. Chemical speciation and formation constant studies show that gallic acid forms a more stable 1:1 complex with zinc ion in comparison to the dihydroxycoumarins.

* Corresponding author. Fax: +81 96 371 4620.
E-mail address: motsuka@gpo.kumamoto-u.ac.jp (M. Otsuka).

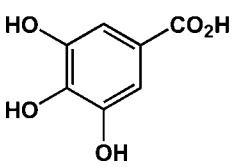
0045-2068/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2004.12.001

Keywords: NF-κB inhibitors; 7,8-Dihydroxy-4-methylcoumarin; 5,7-Dihydroxy-4-methylcoumarin; Gallic acid; p50 protein

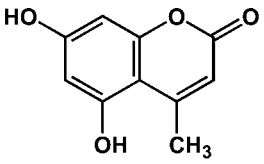
1. Introduction

The design, synthesis, and evaluation of new potential therapeutic agents for the treatment of acquired immunodeficiency syndrome (AIDS) is a significant challenge now facing the medical scientific community. Virtually all the compounds, that are currently used, or under advanced clinical trial, for the treatment of HIV infections have shown certain problems, including that of resistance due to viral mutation [1–3]. Thus, there is a clear need for the development of new antiviral agents that affect unique targets, but do not demonstrate cross-resistance with existing drugs. Nuclear factor-kappa B (NF-κB), an inducible eucaryotic transcription factor of the *Rel* family, normally exists in the form of an inactive cytoplasmic complex, whose predominant form is a heterodimer composed of p50 and p65 (*Rel* A) subunits, bound to inhibitory proteins of the IκB family [4–6]. Activation of NF-κB by a range of stimuli such as microbial and viral products and physical and chemical stresses leads to phosphorylation and proteosome-dependent degradation of IκB, leading to release of free NF-κB [7,8]. This free NF-κB translocates into the nucleus where it binds to the target sites (κB sites) in the DNA to initiate transcription [9]. These κB sites are also present in the long terminal repeat (LTR) of the HIV-1 and hence NF-κB binding to LTR-DNA is critical in viral replication [10]. The strong dependence of the HIV gene expression on NF-κB has made it an important and potential drug target. Further, targeting NF-κB evades the problem of resistance, as it is a normal part of the T-cell and is not subject to mutations. Recent studies have shown that the p50 unit of NF-κB is the one that mainly interacts with HIV-1 LTR [11,12]. The specific amino acids that are classified as the binding site for DNA are residues 59–71 of the p50 subunit of NF-κB (59: Arg, Tyr, Val, Cys, Glu, Gly, Pro, Ser, His, Gly, Gly, Leu, Pro: 71) [13,9]. This particular sequence has been classified as the binding sequence and hereby referred to as the DNA-binding region (DBR). Therefore, targeting direct p50–DNA binding, in this regard is a novel approach to design anti-HIV gene expression inhibitors.

Recently it was reported that polyhydroxycarboxylates derived from phenolic compounds have been found to inhibit the cytopathicity of HIV-1 and HIV-2 in MT-4 cells at concentrations that are not toxic to the host cells [14]. The coumarin derivatives are also reported to be useful in the development of therapies for the treatment of viral infections and diseases, including AIDS [15,16]. Considering the non-toxic nature of these compounds, we have investigated the inhibitory effect of the polyhydroxycarboxylates; 7,8-dihydroxy-4-methylcoumarin (7,8-DHMC), 5,7-dihydroxy-4-methylcoumarin (5,7-DHMC), and gallic acid on recombinant p50 protein–DNA binding with the objective of further designing an inhibitor and also to see how the structures of these compounds relate to their activities (Fig. 1). Prasad et al. [17] have shown that zinc plays an important role in the activation of NF-κB. Therefore,

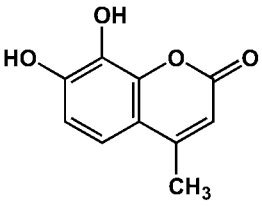


Gallic Acid



5,7-DHMC

(5,7-Dihydroxy-4-methylcoumarin)



7,8-DHMC

(7,8-Dihydroxy-4-methylcoumarin)

Fig. 1. Structures of the compounds.

considering the possible zinc-binding of polyphenolic carboxylate, we have studied the effect of Zn^{2+} ions on the inhibitory activity of gallic acid and have also determined the stability constant of the zinc complexes of the three compounds; 7,8-DHMC, 5,7-DHMC, and gallic acid.

2. Results

2.1. Inhibition of DNA binding of $(p50)_2$

The present study has concentrated on the effect of the polyhydroxycarboxylates: 7,8-DHMC, 5,7-DHMC, and gallic acid, on the p50 protein–DNA binding. Fig. 2 shows the inhibitory effect of coumarins and gallic acid on the DNA binding of $(p50)_2$ at 200 and 500 μM concentrations, as demonstrated by electrophoretic mobility shift assay (EMSA). It was carried out by using chemiluminescence method, which offers the sensitivity and speed without producing the hazardous radioactive waste. Gallic acid was found to inhibit the p50–DNA binding at 200 μM concentration. On the other hand, it was observed that 5,7-DHMC was less potent inhibitor compared with gallic acid. 7,8-DHMC leads to partial inhibition even at 500 μM concentration. Inhibition of the p50–DNA binding by gallic acid was shown to be partly blocked by the addition of one equivalent of Zn^{2+} ions as seen in Fig. 2.

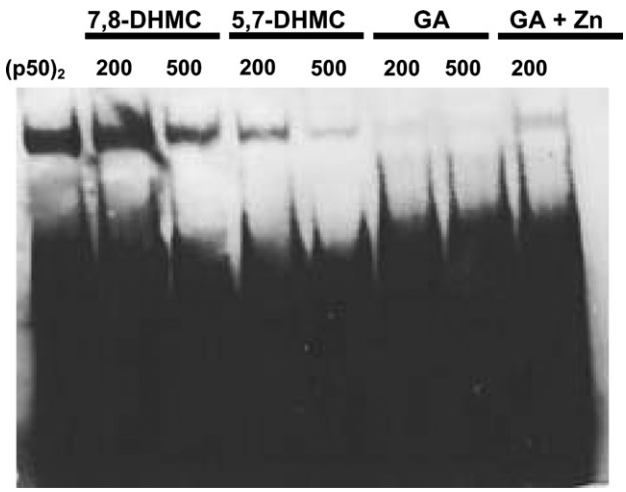


Fig. 2. Effect of coumarins and gallic acid on the DNA binding of $(p50)_2$. All concentrations indicated above are in micromolar unit.

It was considered that these compounds form hydrogen bonds between the amino acid residue of DBR of p50 to exhibit the inhibitory activity. To account for the structure–activity relationship of these compounds, molecular modeling studies were carried

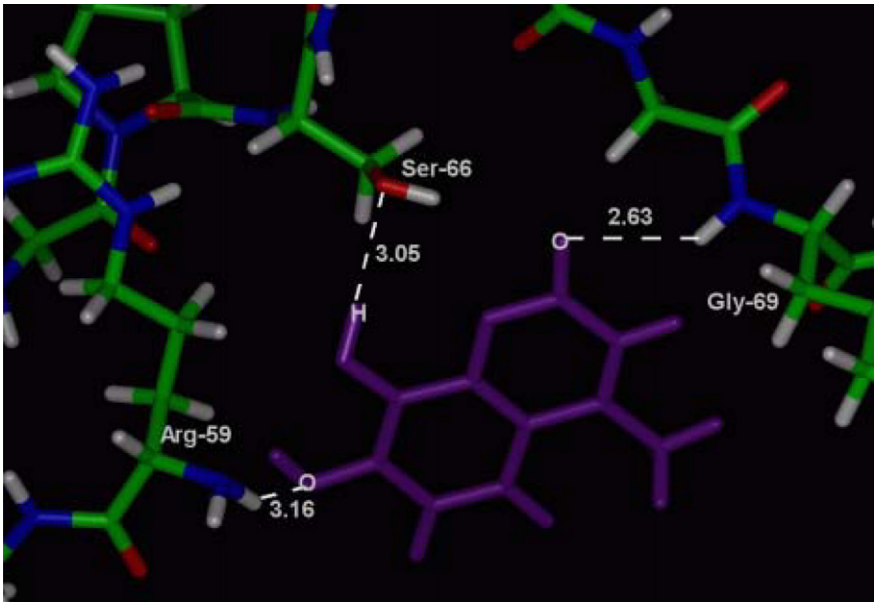


Fig. 3. Hydrogen-bonding interactions of 7,8-DHMC with the DNA-binding region of p50. 7,8-DHMC is shown in purple color. In the DBR, oxygen atoms are shown in red, nitrogen atoms in blue, and hydrogen atoms are shown in white. The atoms of 7,8-DHMC, involved in hydrogen bonds are indicated in letters. All the hydrogen bonds indicated are in Angstrom units.

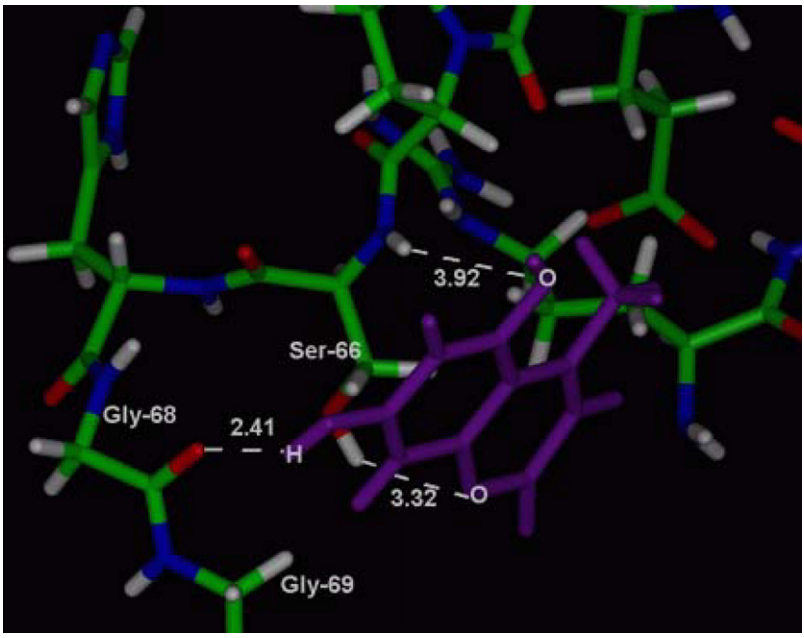


Fig. 4. Hydrogen-bonding interactions of 5,7-DHMC with the DNA-binding region of p50. 5,7-DHMC is shown in purple color. In the DBR, oxygen atoms are shown in red, nitrogen atoms in blue, and hydrogen atoms are shown in white. The atoms of 5,7-DHMC, involved in hydrogen bonds are indicated in letters. All the hydrogen bonds indicated are in Angstrom units.

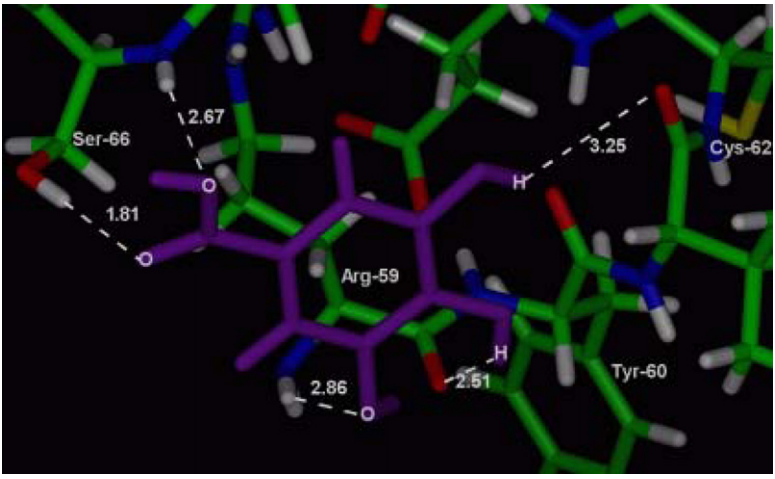


Fig. 5. Hydrogen-bonding interactions of gallic acid with the DNA-binding region of p50. Gallic acid is shown in purple color. In the DBR, oxygen atoms are shown in red, nitrogen atoms in blue, and hydrogen atoms are shown in white. The atoms in gallic acid, involved in hydrogen bonds are indicated in letters. All the hydrogen bonds indicated are in Angstrom units.

out. The calculated interactions of 7,8-DHMC, 5,7-DHMC, and gallic acid with the DBR of p50 are depicted in Figs. 3–5, respectively. Whereas 7,8-DHMC and 5,7-DHMC form three hydrogen bonds with DBR, gallic acid makes five hydrogen bonds, with Ser-66 and the peptide backbone ranging from Arg-59 to Ser-66. Thus, gallic acid interacts more strongly with the DBR than 7,8-DHMC and 5,7-DHMC that make lesser weaker hydrogen bonds. Reversal of inhibition by the addition of zinc could be accounted for by invoking the zinc chelate formation of gallic acid leading to the disruption of the hydrogen bonds with DBR.

2.2. Chemical speciation and formation constant studies

We have employed the versatile pH-metric technique to study the interaction of 7,8-DHMC, 5,7-DHMC, and gallic acid with Zn^{2+}. The data in Tables 1–3 include pH-metric titration data for the calculation of protonation constants of 7,8-DHMC, 5,7-DHMC, and gallic acid. The protonation constants have been calculated using PKAS program [18]. The pH-metric titration data for the calculation of equilibrium constant for 1:1 complexes of the three compounds with Zn^{2+} are shown in Tables 4–6. All for-

Table 1
Titration data for calculation of protonation constants of 7,8-dihydroxy-4-methylcoumarin

TMAH (ml)	pH	TMAH (ml)	pH	TMAH (ml)	pH
0.0	3.28	2.7	9.90	5.4	10.86
0.1	3.34	2.8	10.04	5.5	10.87
0.2	3.38	2.9	10.13	5.6	10.88
0.3	3.43	3.0	10.20	5.7	10.89
0.4	3.50	3.1	10.28	5.8	10.91
0.5	3.58	3.2	10.32	5.9	10.92
0.6	3.69	3.3	10.37	6.0	10.93
0.7	3.83	3.4	10.41	6.1	10.94
0.8	3.99	3.5	10.46	6.2	10.95
0.9	4.18	3.6	10.50	6.3	10.96
1.0	4.39	3.7	10.54	6.4	10.98
1.1	4.64	3.8	10.57	6.5	10.98
1.2	4.84	3.9	10.60	6.6	10.99
1.3	5.05	4.0	10.62	6.7	11.00
1.4	5.21	4.1	10.64	6.8	11.01
1.5	5.35	4.2	10.66	6.9	11.02
1.6	5.49	4.3	10.69	7.0	11.03
1.7	5.67	4.4	10.71		
1.8	5.84	4.5	10.73		
1.9	6.03	4.6	10.75		
2.0	6.44	4.7	10.77		
2.1	7.19	4.8	10.78		
2.2	8.08	4.9	10.79		
2.3	8.70	5.0	10.81		
2.4	9.19	5.1	10.82		
2.5	9.48	5.2	10.83		
2.6	9.70	5.3	10.85		

TMAH, tetramethylammonium hydroxide.

Table 2
Titration data for calculation of protonation constants of 5,7-dihydroxy-4-methylcoumarin

TMAH (ml)	pH	TMAH (ml)	pH	TMAH (ml)	pH
0.0	3.27	2.7	9.76	5.4	10.81
0.1	3.30	2.8	9.90	5.5	10.82
0.2	3.38	2.9	10.00	5.6	10.84
0.3	3.43	3.0	10.11	5.7	10.85
0.4	3.50	3.1	10.19	5.8	10.86
0.5	3.58	3.2	10.23	5.9	10.87
0.6	3.71	3.3	10.29	6.0	10.88
0.7	3.77	3.4	10.34	6.1	10.89
0.8	3.97	3.5	10.38	6.2	10.90
0.9	4.14	3.6	10.43	6.3	10.92
1.0	4.38	3.7	10.46	6.4	10.93
1.1	4.63	3.8	10.49	6.5	10.94
1.2	4.87	3.9	10.51	6.6	10.95
1.3	5.02	4.0	10.54	6.7	10.96
1.4	5.18	4.1	10.58	6.8	10.97
1.5	5.32	4.2	10.60	6.9	10.98
1.6	5.47	4.3	10.62	7.0	10.99
1.7	5.67	4.4	10.64	7.1	11.00
1.8	5.82	4.5	10.66		
1.9	6.00	4.6	10.68		
2.0	6.30	4.7	10.70		
2.1	7.13	4.8	10.73		
2.2	8.10	4.9	10.75		
2.3	8.70	5.0	10.77		
2.4	9.11	5.1	10.78		
2.5	9.36	5.2	10.79		
2.6	9.58	5.3	10.80		

TMAH, tetramethylammonium hydroxide.

mation constants of the 1:1 metal–ligand complexes were obtained with program BEST [18]. Potentiometric equilibrium measurement data of the ligand solution in the presence of metal ion are tabulated in Table 7. The species distribution was determined using the program SPE [18]. The species distribution plots are shown in Figs. 6–8.

3. Discussion

EMSA studies show that gallic acid is more potent inhibitor of p50–DNA binding compared with the two coumarins. The models obtained from docking and optimization showed several interesting results, correlating the structure of these compounds with their respective activities. Biochemical studies have hinted at a relationship between the structural conformation of p50 with the DNA-binding ability and function [19]. Hence, it is plausible that if the conformation of the p50 is affected, its DNA-binding ability may also get affected. From the modeling studies, it seems that among the three compounds, although all of them are making a general steric

Table 3
Titration data for calculation of protonation constants of gallic acid

TMAH (ml)	pH	TMAH (ml)	pH	TMAH (ml)	pH
0.0	2.34	2.7	9.66	5.4	11.55
0.1	2.37	2.8	10.13	5.5	11.57
0.2	2.39	2.9	10.39	5.6	11.58
0.3	2.43	3.0	10.56	5.7	11.60
0.4	2.45	3.1	10.73	5.8	11.62
0.5	2.50	3.2	10.83	5.9	11.63
0.6	2.53	3.3	10.89	6.0	11.65
0.7	2.57	3.4	10.97		
0.8	2.61	3.5	11.01		
0.9	2.64	3.6	11.09		
1.0	2.69	3.7	11.13		
1.1	2.74	3.8	11.17		
1.2	2.80	3.9	11.22		
1.3	2.89	4.0	11.25		
1.4	2.96	4.1	11.28		
1.5	3.06	4.2	11.32		
1.6	3.16	4.3	11.34		
1.7	3.29	4.4	11.36		
1.8	3.55	4.5	11.39		
1.9	3.79	4.6	11.42		
2.0	4.04	4.7	11.43		
2.1	4.45	4.8	11.46		
2.2	4.81	4.9	11.48		
2.3	7.38	5.0	11.49		
2.4	8.18	5.1	11.51		
2.5	8.65	5.2	11.52		
2.6	9.05	5.3	11.54		

TMAH, tetramethylammonium hydroxide.

hindrance (due to van der Waals interactions) for the p50 to bind to its target DNA, gallic acid could also affect the conformation of the protein by making a network of hydrogen bonds with the aid of its uniformly spread hydroxyl group. On the other hand coumarins, though active, have less possibilities of making hydrogen-bonding interactions with the protein. The structure of these compounds could be thus correlated with their activities.

We found that the addition of zinc ion (1 equivalent) partially restored the DNA-binding property of p50. This observation is consistent with Zabel's finding that addition of zinc ion blocked the *o*-phenanthroline inhibition of DNA–NF-κB binding [20]. Prasad et al. [17] have also shown that zinc plays an important role in the activation of NF-κB. Chemical speciation and formation constant studies show that gallic acid forms a stronger 1:1 complex with zinc at physiological pH in comparison to the dihydroxycoumarins. This strong complexation behavior of gallic acid towards zinc may be correlated with its inhibitory activity towards DNA–NF-κB binding.

Table 4
Titration data for Zn^{2+}–7,8-dihydroxy-4-methylcoumarin complex

TMAH (ml)	pH	TMAH (ml)	pH	TMAH (ml)	pH
0.0	3.24	2.7	7.96	5.4	10.54
0.1	3.28	2.8	8.13	5.5	10.57
0.2	3.35	2.9	8.22	5.6	10.59
0.3	3.41	3.0	8.31	5.7	10.61
0.4	3.51	3.1	8.40	5.8	10.63
0.5	3.59	3.2	8.56	5.9	10.64
0.6	3.70	3.3	8.72	6.0	10.65
0.7	3.83	3.4	9.02	6.1	10.67
0.8	3.99	3.5	9.36	6.2	10.68
0.9	4.22	3.6	9.54	6.3	10.69
1.0	4.41	3.7	9.70	6.4	10.71
1.1	4.60	3.8	9.83	6.5	10.73
1.2	4.85	3.9	9.93	6.6	10.75
1.3	4.99	4.0	10.02	6.7	10.77
1.4	5.14	4.1	10.09	6.8	10.78
1.5	5.31	4.2	10.14	6.9	10.79
1.6	5.46	4.3	10.19	7.0	10.80
1.7	5.59	4.4	10.24		
1.8	5.79	4.5	10.28		
1.9	6.00	4.6	10.32		
2.0	6.26	4.7	10.35		
2.1	6.66	4.8	10.38		
2.2	6.98	4.9	10.42		
2.3	7.21	5.0	10.45		
2.4	7.39	5.1	10.48		
2.5	7.60	5.2	10.50		
2.6	7.73	5.3	10.52		

TMAH, tetramethylammonium hydroxide.

4. Experimental

4.1. GST-p50 bacterial protein preparation

In GST–p50 the *Fsp*I–*Apa*I fragment of p105 NF-κB cDNA, which covers the first 464 amino acids, was inserted into pGEX. *Escherichia coli* strain BL21 was transformed with each plasmid, and GST fusion proteins were purified using glutathione–Sepharose column chromatography as described previously by Inoue et al. [21,22].

4.2. Electrophoretic mobility shift assay

7,8-DHMC was synthesized in our laboratory by the Pechmann condensation [23]. 5,7-DHMC and gallic acid were obtained commercially from Koch-Light Laboratories, England, and Thomas Baker, respectively. All the materials used were of reagent grade and were used without purification unless notified. Biotinated double-

Table 5
Titration data for Zn^{2+}–5,7-dihydroxy-4-methylcoumarin complex

TMAH (ml)	pH	TMAH (ml)	pH	TMAH (ml)	pH
0.0	3.31	2.7	7.92	5.4	10.49
0.1	3.34	2.8	8.00	5.5	10.52
0.2	3.38	2.9	8.08	5.6	10.54
0.3	3.44	3.0	8.20	5.7	10.56
0.4	3.53	3.1	8.36	5.8	10.58
0.5	3.61	3.2	8.58	5.9	10.60
0.6	3.72	3.3	8.87	6.0	10.62
0.7	3.87	3.4	9.13	6.1	10.64
0.8	4.03	3.5	9.35	6.2	10.65
0.9	4.22	3.6	9.54	6.3	10.66
1.0	4.47	3.7	9.77	6.4	10.67
1.1	4.64	3.8	9.82	6.5	10.69
1.2	4.85	3.9	9.91	6.6	10.70
1.3	5.01	4.0	9.98	6.7	10.71
1.4	5.15	4.1	10.05	6.8	10.72
1.5	5.32	4.2	10.12	6.9	10.73
1.6	5.43	4.3	10.17	7.0	10.74
1.7	5.63	4.4	10.21	7.1	10.75
1.8	5.78	4.5	10.25		
1.9	5.98	4.6	10.29		
2.0	6.30	4.7	10.33		
2.1	6.68	4.8	10.36		
2.2	7.47	4.9	10.38		
2.3	7.58	5.0	10.41		
2.4	7.67	5.1	10.43		
2.5	7.75	5.2	10.45		
2.6	7.83	5.3	10.47		

TMAH, tetramethylammonium hydroxide.

stranded oligonucleotide containing a κB site from the mouse immunoglobulin κ light chain enhancer

5′-Biotin-AGCTTCAGAGGGGACTTTCCGAGAGG-3′
3′-AGTCTCCCCTGAAAGGCTCTCCAGCT-Biotin-5′

was used. Five nanograms of purified GST–p50 was used for EMSA. After incubation of each reaction mixture containing binding buffer [15 mM Tris–HCl (pH 7.5), 75 mM NaCl, 1.5 mM EDTA, 1.5 mM dithiothreitol, 7.5% glycerol, 0.3% NP-40, and 1 mg/ml BSA], 0.5 μg of poly(dI–dC), GST–p50, and each compound at room temperature for 5 min, labeled DNA probe (30,000 cpm) was added and the mixture was further incubated at room temperature for 20 min. The sample in a volume of 10 μl was loaded onto 4% poly(acrylamide) gels and electrophoresed at 80 CV. After this the gel was transferred to nylon membrane. The biotin end-labeled DNA is detected using the streptavidin–horseradish peroxidase conjugate and LightShift chemiluminescent substrate [24].

Table 6
Titration data for Zn^{2+}–gallic acid complex

TMAH (ml)	pH	TMAH (ml)	pH	TMAH (ml)	pH
0.0	2.35	2.7	6.01	5.4	11.37
0.1	2.36	2.8	6.07	5.5	11.40
0.2	2.40	2.9	6.22	5.6	11.42
0.3	2.41	3.0	6.43	5.7	11.43
0.4	2.43	3.1	6.58	5.8	11.44
0.5	2.46	3.2	6.89	5.9	11.47
0.6	2.49	3.3	7.56	6.0	11.49
0.7	2.54	3.4	8.96		
0.8	2.56	3.5	9.76		
0.9	2.66	3.6	10.17		
1.0	2.72	3.7	10.39		
1.1	2.76	3.8	10.56		
1.2	2.79	3.9	10.67		
1.3	2.87	4.0	10.77		
1.4	2.94	4.1	10.89		
1.5	3.03	4.2	10.94		
1.6	3.13	4.3	11.01		
1.7	3.25	4.4	11.05		
1.8	3.43	4.5	11.10		
1.9	3.70	4.6	11.13		
2.0	4.01	4.7	11.17		
2.1	4.26	4.8	11.20		
2.2	4.68	4.9	11.25		
2.3	5.12	5.0	11.27		
2.4	5.63	5.1	11.31		
2.5	5.85	5.2	11.33		
2.6	5.95	5.3	11.53		

TMAH, tetramethylammonium hydroxide.

Table 7
Overall equilibrium constants for Zn^{2+}–ligand complexes

	Log β HL	Log β H_2L	Log β H_3L	Log β ML	σ_{fit}
Zn(II)–7,8-DHMC	10.75	19.67	—	6.15	0.024724
Zn(II)–5,7-DHMC	10.38	19.51	—	5.79	0.134
Zn(II)–gallic acid	11.48	20.88	25.01	8.71	0.124

DHMC, dihydroxy-4-methylcoumarin.

4.3. Molecular modeling

In the present study, three dimensional structure of NF-κB p50 homodimer bound to a κB site was obtained from the protein data bank (PDB code:1NFK) and analyzed in the QUANTA [25] package.

The molecular structures of the compounds were built in GAUSSVIEW [26a] and optimized at the semiempirical level, using the PM3 Hamiltonian including the molecular mechanics correction for HCON linkages (PM3MM). The calculations were performed using the GAUSSIAN 98 package and analyzed in MOLDEN

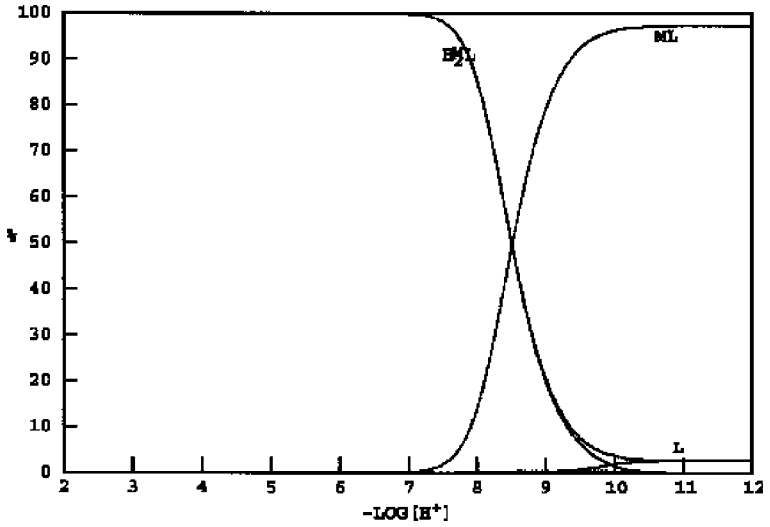


Fig. 6. Species distribution curve for 7,8-DHMC and its zinc complex.

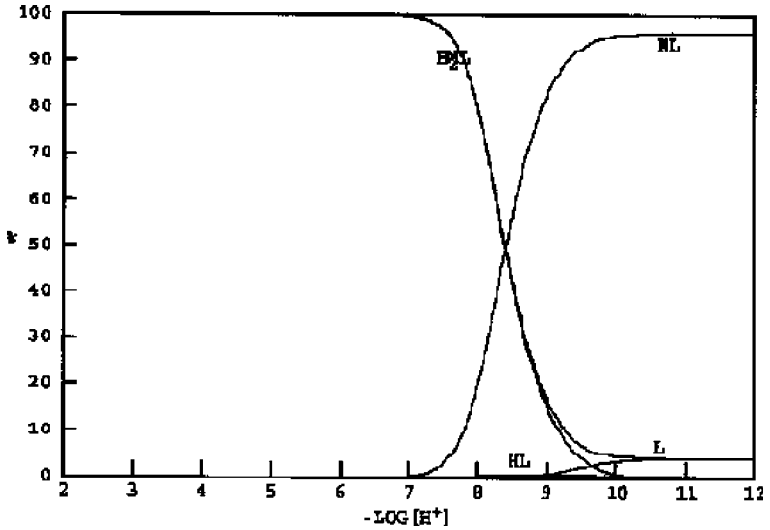


Fig. 7. Species distribution curve for 5,7-DHMC and its zinc complex.

[26b,26c]. These optimized structures were subjected to docking onto the p50 protein, with the amino acids 59–71 (which make the DBR), defined as the docking site. The docking was performed using GOLD 2.0 package, which employs a genetic algorithm for docking flexible ligands onto the protein-binding sites [27]. The interpretation of the docking results is based on the fitness function in the form of scoring (Gold Score). This fitness function is made up of four components, viz.; protein–ligand hydrogen bond energy, protein–ligand van der Waals energy, ligand internal

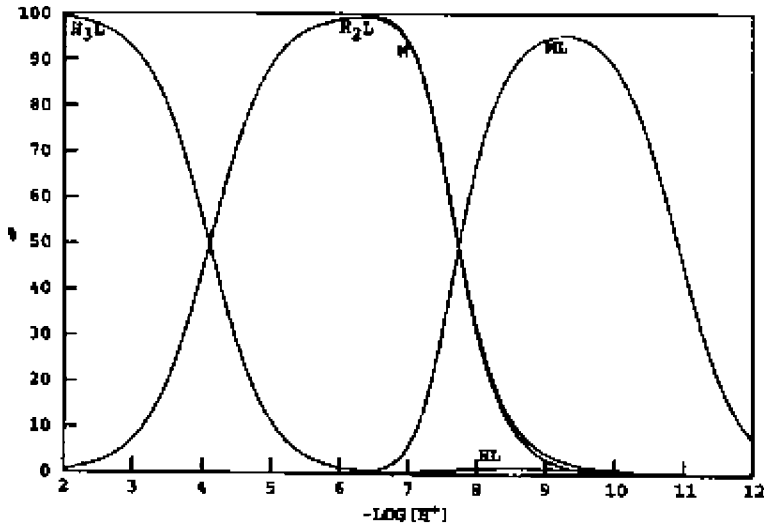


Fig. 8. Species distribution curve for gallic acid and its zinc complex.

van der Waals energy, and ligand tensional strain energy. The best scored docking solution for each compound was considered. The docking solution incorporated the whole p50 protein, with the compound docked in the DBR. Hence, for further analyses, the geometry of the compound, docked with the part of p50 protein exclusively containing the DBR was extracted. This interactive system (compound bound to the DBR), for each compound was subjected to optimization by keeping the protein backbone fixed, using the PM3MM method, as described above.

4.4. Chemical speciation and formation constant studies on Zn^{2+}-polyhydroxycarboxylate complexes

pH-metric technique is a robust and versatile way of measuring ionization and distribution of drugs in biological fluids, and assessing their interaction with trace metal ions. pH-metric titration has been carried out with the digital pH-meter ELICO LI 120 with a combined glass electrode. Glass electrode was calibrated before the titrations as described by Martell and Motekaitis [18]. To ensure constant ionic strength (0.1 M) during the titrations, an electrolyte, sodium nitrate, $NaNO_3$ was added in requisite amounts. A solution of tetramethylammonium hydroxide (TMAH) (E. Merck) in DMF/water was used as the titrant.

Zinc ion solution was prepared from Analar (BDH), samples of the zinc sulfate and was standardized by the conventional methods as described by Vogel [28]. The titrations were performed in a covered glass jacketed titration cell under a stream of presaturated nitrogen. Measurements for 7,8-DHMC and 5,7-DHMC were made in 50% DMF/water medium at 25 °C (±0.5 °C) maintained constant by using Julabo VC type thermostat. For gallic acid, the measurements were made in aqueous medium at 25 °C (±0.5 °C). Solution concentrations of the ligand in the presence and absence of zinc

ions were in the order of 10^{-2} M. Stepwise dissociation constants within the range of the potentiometric titrations (up to pH 12.0) were calculated by fitting the pH data with the help of the program PKAS [18]. Formation constants of the complexes were determined by direct potentiometric titration using the program BEST [18]. Correction factor [29] were applied for the glass electrode in aqueous *N*,*N*-dimethylformamide solutions. A Pentium computer was used for computing the results.

Acknowledgments

M.J.R. thanks NFCR Centre for Computational Drug Design University of Oxford, UK, for a doctoral scholarship to Vineet Pande, Fundação para a Ciência e Tecnologia, Portugal, for financial support. R.K.S. thanks JSPS (Japan) and CSIR (India) for financial assistance.

References

[1] J. Cohen, Science 277 (1997) 32.
[2] J.K. Wong, M. Hezareh, H.F. Gunthard, D.V. Havlir, C.C. Ignacio, C.A. Spina, D.D. Richman, Science 278 (1997) 1291.
[3] D. Finzi, M. Hermankova, T. Pierson, L.M. Carruth, C. Buck, R.E. Chaisson, T.C. Quinn, K. Chadwick, J. Margolick, R. Brookmeyer, J. Gallant, M. Markowitz,, D.D. Ho, D.D. Richman, R.F. Siliciano, Science 278 (1997) 1295.
[4] P.A. Baeuerle, T. Henkel, Annu. Rev. Immunol. 12 (1994) 141.
[5] A. Israel, Trends Cell Biol. 10 (2000) 129.
[6] S. Ghosh, M.J. May, E.B. Kopp, Annu. Rev. Immunol. 16 (1998) 225.
[7] R.I. Scheinmann, P.G. Cogswell, A.K. Lofquist, A.S. Baldwin, Science 270 (1995) 283.
[8] H.M. Lander, J.S. Ogiste, S.F. Pearce, R. Levi, A. Novogrodsky, J. Biol. Chem. 270 (1995) 7071.
[9] B. Berkowitz, D.-B. Huang, F.E. Chen-Park, P.B. Sigler, G. Ghosh, J. Biol. Chem. 277 (2002) 24694.
[10] G. Nabel, D. Baltimore, Nature 326 (1987) 711.
[11] J. Hiscott, H. Kwon, P. Genin, J. Clin. Invest. 107 (2001) 143.
[12] L.A. Dickinson, J.W. Trauger, E.E. Baird, P.B. Dervan, B.J. Graves, J.M. Gottesfeld, J. Biol. Chem. 274 (1999) 12765.
[13] C.W. Muller, F.A. Rey, M. Sodeoka, G.L. Verdine, S.C. Harrison, Nature 373 (1995) 311.
[14] D. Schols, P. Wutzler, R. Kloecking, B. Helbig, E. De Clercq, J. Acquired Immune Def. Syndr. 4 (1991) 677.
[15] J.M. Domagala, S.E. Hagen, E. Lunney, B.D. Tait, (Warner-Lambert Co., USA). U.S. (1996), 17 pp. CODEN: USXXAM US 5510375 A 9960423 Patent written in English. Application: US 93-155728 19931119. CAN 125:33478 AN 1996: 323769.
[16] J.G. Park, J.C. Park, J.M. Hur, S.J. Park, D.R. Choi, D.Y. Shin, K.Y. Park, H.W. Cho, M.S. Kim, Nat. Prod. Sci. 6 (2000) 117.
[17] A.S. Prasad, B. Bao, F.W.J. Beck, F.H.J. Sarkar, Lab. Clin. Med. 138 (2001) 250.
[18] A.E. Martel, R.J. Motekaitis, In Determination and Use of Stability Constants, VCH Publishers, New York, 1992.
[19] F.E. Chen-Park, D.-B. Huange, B. Noro, D. Thanos, G. Ghosh, J. Biol. Chem. 277 (2002) 24701.
[20] U. Zabel, R. Schreck, P.A. Baeuerle, J. Biol. Chem. 266 (1991) 252.
[21] J. Inoue, L.D. Kerr, A. Kakizuka, I.M. Verma, Cell 68 (1992) 1109.
[22] J. Inoue, T. Takahara, T. Akizawa, O. Hino, Oncogene 8 (1993) 2067.
[23] H.V. Pechmann, J.B. Cohen, Ber. 17 (1884) 2187.

[24] Pierce Biotechnology, Inc., Rockford IL, USA.
[25] QUANTA 97 (Version 97. 0711), Molecular Simulations Incorporated.
[26] (a)GAUSSVIEW. Gaussian Inc., Carnegie office park, Bldg.6, Pittsburgh, PA 15106, USA(b)M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, V.G. Zakrzewski, J.A. Montgomery, R.E. Stratmann, J.C. Burant, S. Dapprich, J.M. Millam, A.D. Daniels, K.N. Kudin, M.C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G.A. Petersson, P.Y. Ayala, Q. Cui, K. Morokuma, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J. Cioslowski, J.V. Oritz, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P.M.W. Gill, B.G. Johnson, W. Chen, M.W. Wong, J.L. Andres, M. Head-Gordon, E.S. Replogle, J.A. Pople, Gaussian 98 (Revision A1), Gaussian Inc., Pittsburgh, PA, 1998;
(c)G. Schaftenar, J.H. Noordik, J. Comp.–Aided Mol. Des. 14 (2000) 123.
[27] GOLD 2.0 (CDC Software Limited) G. Jones, P. Willett, R.C. Glen, A.R. Leach, R. Taylor, J. Mol. Biol. 267 (1997) 727.
[28] V.A. Vogel, in: Text Book of Quantitative Inorganic Analysis, fourth ed., Longmans, ELBS, New York, 1978.
[29] G. Ganzalez, D.G. Resales, J. Ariza, P. Land, Talanta 33 (1986) 105.