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Evaluation of the interaction potential of synthetic ethylene glycol compounds with nuclear Factor κB

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Abstract: In the last three decades, nuclear factor κB (NF-κB) has been the focus of many researchers who are interested in understanding the various molecular mechanisms involved in inflammatory diseases and cancer. Interference with NF-κB activity can cause many cellular abnormalities including tumorigenesis. In this study, we focus on examining the potential of ten synthetic ethylene glycol based compounds to interact with the binding site of NF-κB. Computational analysis reveals that the ethylene glycol compounds examined in this study display differential ability to interact with NF-κB. Parameters such as ALOGP, dock score, and internal energy were determined for each of the studied compounds. Seven compounds were found to interact with specific amino acid residues within the binding site of NF-κB. The specific amino acids involved in the interaction were mapped and the nature of interaction was identified. Since NF-κB is implicated in the development of many inflammatory and immune-related conditions including cancer, we predict that the ethylene glycol compounds examined in this study can be targeted for the development of therapeutic agents that can be employed in the prevention and/or treatment of various diseases including cancer.

Keywords: Ethylene Glycol Compounds, Nuclear Factor κB, Anti-inflammation, Anti-cancer.

Introduction

NF-κB comprises a family of ubiquitously expressed, eukaryotic transcription factors that participate in the regulation of multiple immediate genes that are expressed at the onset of many vital biological processes such as cell growth, immunoregulation, apoptosis, and inflammation[1,2]. Modulation of NF-κB activity can lead to many abnormal cellular processes and diseases including asthma, arthritis, atherosclerosis, obesity, and various types of cancers[1-6]. NF-κB exists in cells as a heterodimer of members of the Rel family of proteins, including p50, p52, p65 (RelA), RelB, and c-Rel, which share a high degree of structural similarity[7]. Indeed, NF-κB is present in the cytoplasm of the cell in an inactive form until certain receptors are triggered, upon which it becomes transcriptionally active to turn on the expression of its target genes in a timely-regulated fashion. NF-κB activation can be achieved via the classical or alternative pathways, which are turned on by distinct stimuli and lead to

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different outcomes. NF-κB is a survival, anti-apoptotic factor that has been shown to be persistently active in various types of cancer including those of breast, liver, and colon[7].

Compelling experimental evidence suggesting that NF-κB proteins are essential for the development of a normal, effective immune system arose from genetically-engineered knockout models. Indeed, NF-κB genetic ablation was shown to render mice immune-compromised and prone to pathogenic infections[8-12]. Thus, it is evident that generation of effective inflammatory, innate, and adaptive immune responses against microbial pathogens as well as cancer cells is dependent on coordinated NF-κB activity.

Under basal conditions, most NF-κB subunits are sequestered in the cytosol, where they are constitutively bound by members of the NF-κB inhibitor family of proteins, mainly IκBα and IκBβ[13,14]. However, diverse stimuli, including inflammatory cytokines, mitogens, lipopolysaccharides, UV light, as well as bacterial and viral pathogens can transduce a signal that ultimately results in NF-κB liberation from its inhibitors, allowing NF-κB dimers to translocate to the nucleus and become transcriptionally active[15-17]. Upon stimulation, IκB subunits become phosphorylated, ubiquitinated, and subsequently degraded, allowing NF-κB subunits to translocate to the nucleus and bind as dimers to κB responsive elements of target genes[7]. Dimerization of NF-κB proteins is a prerequisite for NF-κB to become transcriptionally active[15].

Transcription factor NF-κB is widely present in eukaryotic cells as a regulator of genes involved in the control of cell proliferation and cell survival. Any defects in NF-κB's regulation may lead to enhanced apoptosis causing cell death. In addition, NF-κB is a key player in inflammatory responses. Thus, inhibiting NF-κB signaling has potential therapeutic applications in preventing and/or treating inflammatory diseases and different types of cancer.

Drugs like disulfiram, dithiocarbamates and olmesartan, which can serve as potent inhibitors of NF-κB activity, are shown in **Figure 1**. Disulfiram inhibits NF-κB activity by preventing NF-κB nuclear translocation and its DNA binding potential, without affecting IκBα function[18]. Dithiocarbamates inhibit NF-κB activity by blocking its nuclear translocation via preventing IκBα degradation through the ubiquitylation-proteosome proteolytic pathway[19], without influencing NF-κB DNA binding activity[20]. Olmesartan, a blocker of angiotensin II type 1 receptors, shows ethylene glycol ester grouping as a potential pharmacophore, and it inhibits NF-κB activity by preventing its nuclear accumulation[21] and impeding its promoter activity[22].

Ethylene glycol is a colorless organic compound mostly used as an antifreeze chemical in automobiles, air conditioners, and de-icing fluid[23]. Ethylene glycol is known to cause serious kidney toxicity, liver damage, and central nervous system depression[24]. Also, ethylene glycol is known to be a mild destabilizer of proteins as is evident from its ability to decrease the melting temperature (T_m) of proteins[25], and it has been shown to interfere with hydrophobic interactions[26,27].

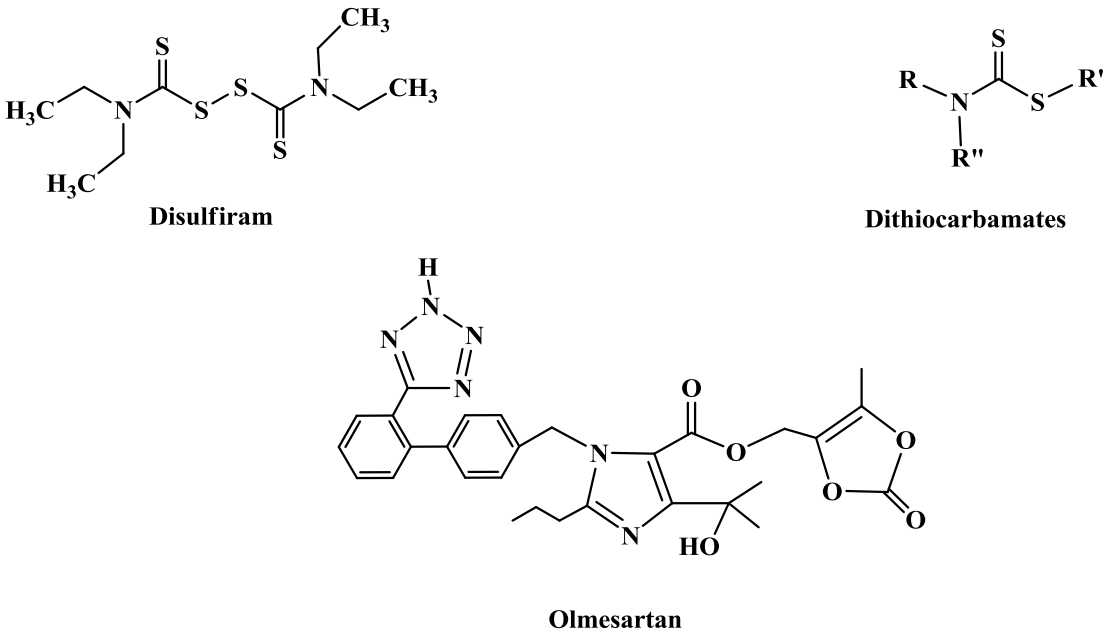
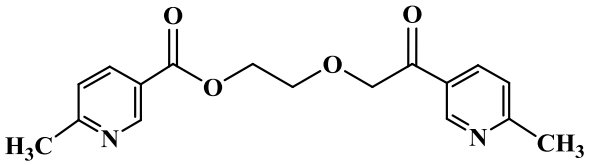


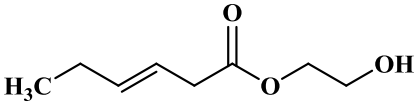
Figure 1: NF-κB inhibitors (disulfiram, olmesartan and dithiocarbamates)

Polyethylene glycol (PEG) is a water-soluble relative of ethylene glycol. It is used for many purposes in biotechnology, drug design, and medicine as it is non-toxic and non-immunogenic. Pegylation, attaching a polyethylene glycol (PEG) moiety, improves the pharmacokinetic and pharmacodynamic profiles of proteins by increasing their half-lives[28]. Modification by polyethylene glycol (PEG) has been reported to be an effective method for improved tumor targeting and drug delivery[29,30]. Interestingly, conjugation of proteins with polyethylene glycol has been reported to be effective in inhibiting tumor growth in mouse models[31].

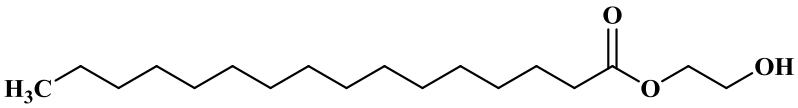
This study is mainly focused on the binding activity of the ethylene glycol derivatives against NF-κB to regulate inflammatory responses and cancer development, using appropriate tools and software to validate theoretically the effective drug candidates. Ten ethylene glycol compounds were examined in this study. The structures of the molecules are shown in **Figure 2** along with their IUPAC names, and their general properties are stated in **Table 1**. Seven of the ten synthetic compounds were found to interact with specific amino acid residues within the binding site of NF-κB. The specific amino acids involved in the interaction were mapped and the nature of the interaction was identified. Given that active NF-κB is critically involved in inducing inflammatory responses and mediating tumor formation, we anticipate that some of the ethylene glycol compounds examined in this study may be employed as effective therapeutic agents in the regulation of diverse immune reactions, implicated in various infectious and non-infectious conditions and diseases, including cancer.



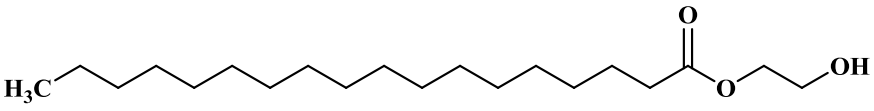
1; 1,2-Bis(2-methylpyridine-4-yl)carbonyl]oxy}ethane



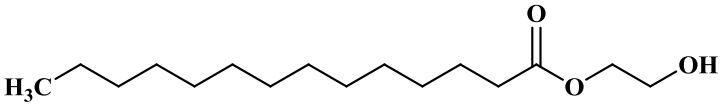
2; 2-Hydroxyethyl-(3E)-hex-3-enoate



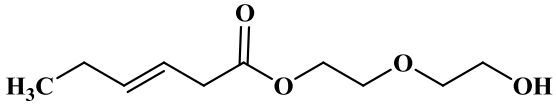
3; 2-Hydroxyethylhexadecanoate



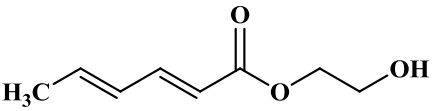
4; 2-Hydroxyethyloctadecanoate



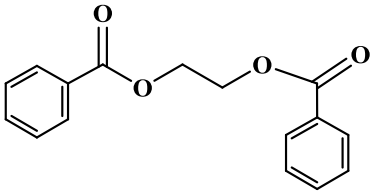
5; 2-Hydroxyethyltetradecanoate



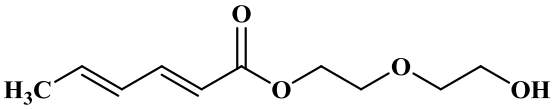
6; 2(2-Hydroxyethoxy)ethyl-(3E)-hex-3-enoate



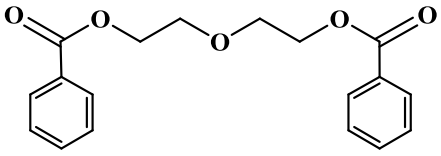
7; 2-Hydroxyethyl-(2E,4E)-hex-2,4-dienoate



8; 1,2-Dibenzoyloxyethane



9; 2-(2-Hydroxyethoxy)ethyl-(2E,4E)-hex-2,4-dienoate



10; O,O-Dibenzoyldiethylene glycol

Figure 2: IUPAC names and structures of ethylene glycol compounds

Table 1: Chemical properties of ethylene glycol compounds

Compound	No of Atoms other than H-Atoms	Molecular Composition	Molecular Formula	Molecular Weight (daltons)
1	22	C: 0.640 H: 0.054 O: 0.213 N: 0.093	$C_{16}H_{16}N_2O_4$	300.318
2	11	C: 0.607 H: 0.089 O: 0.303	$C_8H_{14}O_3$	158.200
3	21	C: 0.719 H: 0.121 O: 0.160	$C_{18}H_{36}O_3$	300.486
4	23	C: 0.731 H: 0.123 O: 0.146	$C_{20}H_{40}O_3$	328.540
5	19	C: 0.705 H: 0.118 O: 0.176	$C_{16}H_{32}O_3$	272.432
6	14	C: 0.594 H: 0.090 O: 0.316	$C_{10}H_{18}O_4$	202.254
7	11	C: 0.615 H: 0.077 O: 0.307	$C_8H_{12}O_3$	156.184
8	20	C: 0.711 H: 0.052 O: 0.237	$C_{16}H_{14}O_4$	270.288
9	14	C: 0.588 H: 0.099 O: 0.313	$C_{10}H_{20}O_4$	204.27
10	23	C: 0.688 H: 0.058 O: 0.254	$C_{18}H_{18}O_5$	314.342

Experimental Section

The ethylene glycol esters were conveniently synthesized by transesterification as shown **Scheme 1**[32].

Scheme 1: General equation for transesterification reactions

The uniprot/swissprot is a useful protein sequence database that can provide invaluable information related to the function of a protein, the structure of a protein domain, post-translational modifications, isoforms, among other things[33]. The uniprot/swissprot database provides a minimal level of redundancy and high level of integration when compared to other databases. The protein data bank (PDB) archive is one of the most important sources of information related to the 3D structures of biological macromolecules such as proteins[34-35]. The massive information stored in this protein data bank archive can help scientists understand the potential role of specific compounds in human health, disease pathophysiology, and drug design. Accelrys Discovery studio 2.1[36] is a software for computational chemistry and biology and is designed for applications in drug development[37]. This product features algorithmic tools for protein modeling and other various solutions for bioinformatics and cheminformatics.

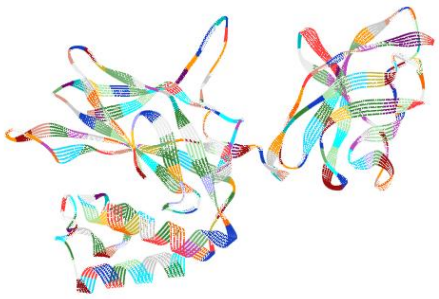


Figure 3: The structure of NF-κB as shown by Discovery Studio Visualizer 2.5, a simulation software that was used to generate and visualize the NF-κB structure.

The structure of NF-κB with PDB ID of 1svc (**Figure 3**) with a resolution factor of 2.60 Å was retrieved from the protein databank as an effective drug target for anti-inflammatory agents. Recent studies have shown that signaling of NF-κB is involved in its activation of inflammation and tumor development.

Results and Discussion

Drug likeness property

Drug likeness properties of ethylene glycol compounds are evaluated using parameters such as ALOGP, molecular weight, hydrogen bond acceptor (HBA), and hydrogen bond donor (HBD) characteristics, as shown in **Table 2**.

Table 2: Drug likeness property of ethylene glycol compounds

Compound	ALOGP	Molecular Weight (daltons)	Hydrogen Bond Acceptor (HBA)	Hydrogen Bond Donor (HBD)
1	1.453	300.309	6	0
2	1.072	158.195	3	1
3	6.079	300.477	3	1
4	6.991	328.53	3	1
5	5.166	272.423	3	1
6	0.941	202.248	4	1
7	1.049	156.179	3	1
8	3.189	270.28	4	0
9	1.386	204.263	4	1
10	3.058	314.333	5	0

In rational drug design, many researchers rely on ALOGP as a very effective computational method in the measuring molecular hydrophobicity (lipophilicity), which is usually quantified as logP (the logarithm of 1-octanol/water partition coefficient)[38-41]. Molecular hydrophobicity reflects on the biological and biochemical properties of drugs, including their lipid solubility, absorption, tissue distribution, bioavailability, receptor interaction, metabolism, cellular uptake, and toxicity[42]. ALOGP is more advantageous over other computational methods that quantify logP (e.g. CLOGP), because it allows for the estimation of local hydrophobicity, the visualization of molecular hydrophobicity maps, and the evaluation of hydrophobic interactions in ligand-receptor complexes[42]. Nowadays, LogP is utilized as a very important parameter in studies of the environmental fate of chemicals and the rate at which chemical degradation occurs. As illustrated in Table 2, our results shown that monoesters of glycol have lower ALOGP values, while the long fatty chain esters have higher ALOGP values in accordance with the lipophilicity of the molecules. Diesters of short chains show intermediate values. Interestingly, the higher lipophilic compounds have shown better activity than the hydrophilic compounds (Table 2).

Lipinski's rule of five, which was formulated by Christopher Lipinski in 1997, is an empirical derivation that is used to assess the drug likeness of chemical compounds that have pharmacological and biological potential to serve as an orally active and potent drug[43]. The formulation of Lipinski's rule of five is based on the observation that orally active drugs are small in size and have optimal solubility in aqueous (i.e. water) and non-polar (e.g. fat) media[43-45]. As per Lipinski's rule of five, a value of ALOGP of $\leq$5, a molecular weight of $\leq$500 amu, a number of hydrogen bonding acceptor sites (HBA) of $\leq$10, a number of hydrogen bonding donor sites (HBD) of $\leq$5 are ideal for a drug[43-45]. In some cases, however, a drug can be deemed suitable even if it violates one of the four criteria of Lipinski's rule of five[43-45]. Thus, considering the potential activity of a drug coupled with *in vivo* assessment of its properties is a constructive strategy in drug design.

Ligand-receptor binding affinity

The ten compounds shown in **Table 1** were subjected to docking studies. The protein was retrieved from the protein databank (PDB) and prepared as per the requirements necessitated by the tools and software used. A sphere was determined for the binding of the lead compound to the receptor binding site (Figure 4). The binding site search was carried out in a shape-based mode of the receptor using the eraser and flood-filling algorithm. From twelve possible binding sites of increasing volumes, site 1 of volume 80.25Å with 642 point count in 3D view direction of X= 40.4 Å, Y= 34.123 Å, Z= 45.039 Å and grid spacing 0.5 x 0.5 x 0.5 surrounded by sphere of radius 5.6 cm was determined. **Figure 4** shows the sphere of site 1 of NF-κB.

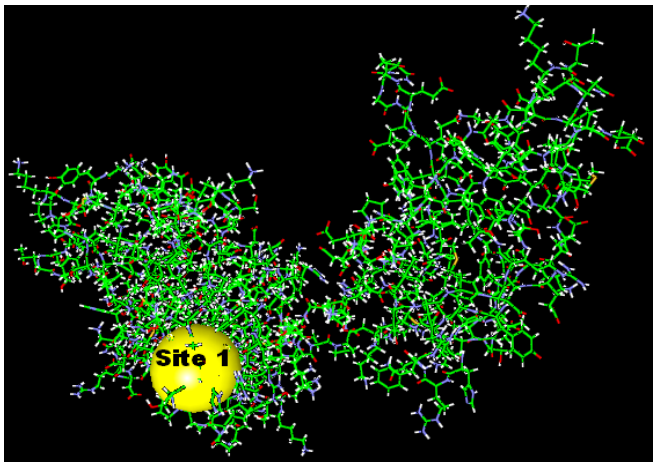


Figure 4: Binding site 1 of NF-κB

After the initial placement of the ligand in the binding site, a rigid body minimization of the ligand was performed using a steepest descent (SD) algorithm, and optionally followed by a Broyden-Fletcher-Goldfarb-Shanno (BFGS) Quasi-Newton minimization. The energy grid computed earlier was used to calculate interactions between the ligand and the receptor**.** Conformations of each analogy were created with Monte Carlo simulation (15,000 trials) and a flexible fit was selected. The RMSD threshold and score threshold were set to 1.5 and 20 kcal⁄mol, respectively, in an attempt to avoid identical conformations. Each of the saved conformations was evaluated and ranked using the internal energy and dock score parameters. The binding energy and dock score values specific to each of the ten ethylene glycol compounds targeting site 1 of NF-κB are shown in **Table 3**.

Aside from determining the internal energy and dock score values, it is necessary to assess the nature of interaction between the ethylene glycol compounds and the amino acid residues within the binding site of NF-κB. **Table 4** shows the nature of interaction and the exact amino acid residues involved. Some amino acid residues were found to play an important role in the binding of inhibitors within the binding site of NF-κB. The amino acids that actively participated in the interaction with ethylene glycol derivatives include Ile142, Asn139, Pro65, and Arg59.

Table 3: Internal energy and dock score values of ethylene glycol compounds

Compound	Dock score	Internal energy (kcal/mol)
1	40.698	-2.791
2	34.615	-1.756
3	50.971	-3.933
4	46.743	-6.615
5	48.014	-3.987
6	41.369	-2.686
7	32.491	-1.416
8	40.641	-2.989
9	39.489	-2.272
10	47.302	-3.609

Interactions between the ethylene glycol compounds and the amino acid residues within the binding site of NF-κB occur mainly through van der Waals forces and hydrogen bonding (**Table 4**). Biochemically, hydrogen bonding represents a strong mode of non-covalent molecular forces that are capable of stabilizing vital interactions between different moieties that even external pressure cannot easily disturb. In contrast, even a minor disturbance may tend to influence the entire interaction potential between moieties that are held by van der Waals forces.

The interaction distances between the ethylene glycol compounds (**3-7, 9**) and the corresponding amino acid residues within the binding site of NF-κB are shown in **Table 5**.

Table 4: Nature of interaction between ethylene glycol compounds and the amino acid residues within the binding site of NF-κB

Compound	Nature of Interaction	Amino Acid
1	Van der Waals	-----
2	Van der Waals	-----
3	Hydrogen	Ile142
4	Hydrogen	Pro65
5	Hydrogen	Asn139
6	Hydrogen	Ile142
7	Hydrogen	Asn139
8	Van der Waals	-----
9	Hydrogen	Arg59, Ile142
10	Van der Waals	-----

Table 5: Interaction distance between compounds **3-7**, **9** and the amino acid residues within the binding site of NF-κB

Compound	Amino Acid	Interaction Distance (Å)
3	Ile142	2.469
4	Pro65	2.483
5	Asn139	3.180
6	Ile142	2.486
7	Asn139	2.912
9	Ile142	2.491
9	Arg59	2.136 2.149

A schematic representation of the chemical interaction, along with the distance, between ethylene glycol compounds **3-7, 9** and the amino acid residues within the binding site of NF-κB is shown in **Figure 5**.

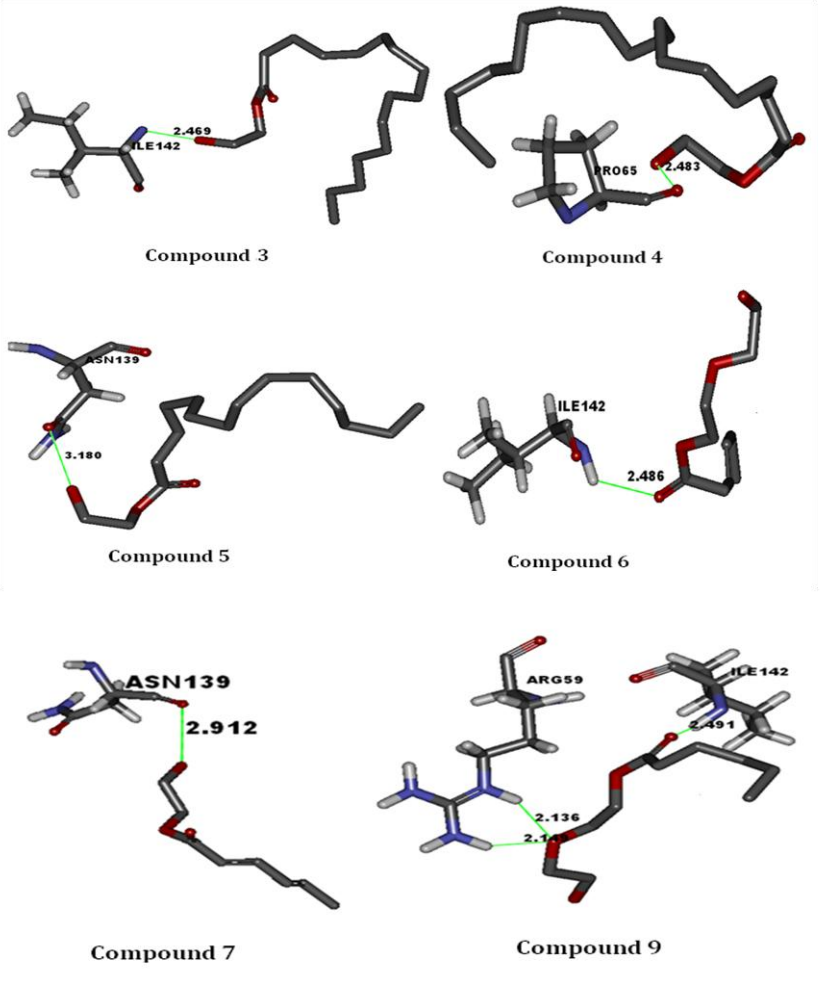


Figure 5: A schematic representation of the chemical interaction, along with the distance, between ethylene glycol compounds **3-7**, **9** and the amino acid residues within the binding site of NF-κB.

One major concern in drug design is the potential molecular interaction between small molecule drugs and non-target proteins expressed in the cell. However, the spatial arrangement of the pharmacophores could bind in a very specific manner to the target proteins. The studied ethylene glycol compounds are derived based on the spatial requirement for the NF-κB site exhibited by proven drugs, and Table 3 illustrates that the interaction between the studied ethylene glycol compounds and NF-κB is strong. Yet, it is still possible that some cellular proteins share a homologous amino acid sequence with NF-κB at the site where ethylene glycol compounds bind, allowing these compounds to potentially interact not only with NF-κB, but also with such homologous non-target proteins and influence other cellular pathways and processes. To examine this possibility, future studies should aim at investigating the potential molecular interaction between the studied ethylene glycol compounds and such non-target proteins.

Conclusion

This study reveals that ethylene glycol compounds possess a strong interaction potential with NF-κB. Among the ten compounds examined in this study, compound **3** has the highest dock score and interaction potential, whereas compound **7** has the lowest dock score and interaction potential. The descending order of the ethylene glycol compounds studied with respect to their dock score values is as follows:

Compound **3** > Compound **5** > Compound **4** > Compound **6** > Compound **9** > Compound **7**

The Lipinski prediction proved to be very effective in the identification of more suitable ligands towards target proteins. The current study shows that the linear aliphatic chain esters (e.g. compounds **3**, **4**, **5**, **6**, **7**, **9**) display very good interaction with NF-κB as compared to phenyl- and pyridyl containing molecules (e.g., compounds **1**, **8**, **10**). We speculate that the presence of one or more aromatic or heteroaromatic rings interferes, most likely due to steric hindrance, with the potential of ethylene glycol derivatives to interact with NF-κB. Since the exact details of the molecular mechanism undertaken by ethylene glycol compounds to interact with NF-κB are not fully understood, more experimental studies are needed to reveal the reason behind the differential potential of linear aliphatic chain esters versus benzene-based molecules to interact with NF-κB. Based on the ALOGP values (Table 2) and on the Lipinski's rule of five (ALOGP ≤5, molecular weight ≤500, HBA ≤10, HBD ≤5)[43-45], we speculate that compounds **6**, **7** and **9** could serve as ideal drug candidates of therapeutic NF-κB inhibitors. *In silico* screening methods are routinely and extensively used to reduce the time and cost associated with drug discovery. Clearly, the approach undertaken in this study is successful in searching for novel ethylene glycol based compounds that are capable of binding, with different degrees, to NF-κB. The synthetic compounds that targeted the NF-κB protein were screened based on the dock score and nature of interaction. Our study sheds light on ethylene glycol compounds that are capable of binding NF-κB, most likely inhibiting its intracellular signaling.

Since NF-κB activity is critically implicated in inflammatory responses as well as immune responses that are generated against microbes and cancer cells, the compounds examined in this study can serve as potential targets for the development of anti-inflammatory and anti-cancer drugs. Hence, we anticipate that some of the ethylene glycol compounds examined in

this study may be employed as effective therapeutic agents in the regulation of diverse immune reactions implicated in various infectious and non-infectious conditions and diseases, including different types of cancer.

Acknowledgements

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References

1. P. P. Tak, G. S. Firestein, J. Clin. Invest., **2001**, *107*, 7-11.
2. Y. Yamamoto, R. B. Gaynor, J. Clin. Invest., **2001**, *107*, 135-142.
3. S. E. Shoelson, J. Lee, M. Yuan, Int. J. Obes. Relat. Metab. Disord., **2003**, *27*, S49-S52.
4. H. Clevers, Cell, **2004**, *118*, 671-674.
5. A. Kumar, Y. Takada, A. M. Boriek, B. B. Aggarwal, J. Mol. Med., **2004**, *82*, 434-448.
6. C. Monaco, E. Paleolog, Cardiovasc. Res., **2004**, *61*, 671-682.
7. A. F. Majdalawieh, H. S. Ro, Mediators Inflamm., **2010**, *2010*, 823821.
8. W. C. Sha, H. C. Liou, E. I. Tuomanen, D. Baltimore, Cell, **1995**, *80*, 321-330.
9. F. Weih, D. Carrasco, S. K. Durham, D. S. Barton, C. A. Rizzo, R. P. Ryseck, S. A. Lira, R. Bravo, Cell, **1995**, *80*, 331-340.
10. F. Kontgen, R. J. Grumont, A. Strasser, D. Metcalf, R. Li, D. Tarlinton, S. Gerondakis, Genes Dev., **1995**, *9*, 1965-1977.
11. J. H. Caamano, C. A. Rizzo, S. K. Durham, D. S. Barton, C. Raventos-Suarez, C. M. Snapper, R. Bravo, J. Exp. Med., **1998**, *187*, 185-196.
12. G. Franzoso, L. Carlson, L. Poljak, E. W. Shores, S. Epstein, A. Leonardi, A. Grinberg, T. Tran, T. Scharton-Kersten, M. Anver, P. Love, K. Brown, U. Siebenlist, J. Exp. Med., **1998**, *187*, 147-159.
13. P. A. Baeuerle, D. Baltimore, Genes Dev., **1989**, *3*, 1689-1698.
14. S. Ghosh, D. Baltimore, Nature, **1990**, *344*, 678-682.
15. M. J. May, S. Ghosh, Semin. Cancer Biol., **1997**, *8*, 63-73.
16. A. Israel, Trends Cell Biol., **2000**, *10*, 129-133.
17. M. S. Hayden, S. Ghosh, Genes Dev., **2004**, *18*, 2195-2224.
18. W. Wang, H. L. McLeod, J. Cassidy, Int. J. Cancer, **2003**, *104*, 504-511.
19. Y. Q. Li, Z. X. Zhang, Y. J. Xu, W. Ni, S. X. Chen, Z. Yang, D. Ma, Acta. Pharmacol. Sin., **2006**, *27*, 339-346.
20. R. Schreck, B. Meier, D. N. Mannel, W. Droge, P. A. Baeuerle, J. Exp. Med., **1992**, *175*, 1181-1194.
21. T. Aoki, H. Kataoka, R. Ishibashi, H. Nakagami, K. Nozaki, R. Morishita, N. Hashimoto, Neurosurgery, **2009**, *64*, 357-366.
22. S. Yamagishi, T. Matsui, K. Nakamura, H. Inoue, M. Takeuchi, S. Ueda, K. Fukami, S. Okuda, T. Imaizumi, Microvasc. Res., **2008**, *75*, 130-134.
23. J. Patocka, Z. Hon, Acta Medica, **2010**, *53*, 19-23.
24. R. Hess, M. J. Bartels, L. H. Pottenger, Arch Toxicol., **2004**, *78*, 671-680.
25. K. Gekko, T. Morikawa, J. Biochem., **1981**, *90*, 51-60.

26. D. Freifelder, Physical Biochemistry Applications to Biochemistry and Molecular Biology, 2nd ed., W.H. Freeman, New York, **1984**.
27. R. H. Al-Shakhshir, F. E. Regnier, J. L. White, S. L. Hem, Vaccine, **1995**, *13*, 41-44.
28. S. L. Yowell, S. Blackwell, Cancer Treatment Rev., **2002**, *28*, S3-S6.
29. H. Wada, I. Imamura, M. Sako, S. Katagiri, S. Tarui, H. Nishimura, Y. Inada, Ann. NY Acad. Sci., **1990**, *613*, 95-108.
30. G. E. Francis, C. Delgado, D. Fisher, F. Malik, A. K. Agrawal, J. Drug Target., **1996**, *3*, 321-340.
31. C. L. van den Berg, G. N. Cox, C. A. Stroh, S. G. Hilsenbeck, C. N. Weng, M. J. McDermott, D. Pratt, C. K. Osborne, E. B. Coronado-Heinsohn, D. Yee, Eur. J. Cancer, **1997**, *33*, 1108-1113.
32. S. Narasimhan, S. Gopi, V. Ramasubramanian, D. P. Matharasi, S. Kannan, Indian Patent Application: 1951/CHE/2009.
33. http://www.uniprot.org
34. H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov, P. E. Bourne, Nucleic Acids Res., **2000**, *28*, 235-242.
35. H. M. Berman, Acta Crystallographica Section A: Foundations of Crystallography, **2008**, *A64*, 88-95.
36. http://accelrys.com/resource-center/downloads/updates/discovery-studio/dstudio25/dstudio255.html
37. C. M. Venkatachalam, X. Jiang, T. Oldfield, M. Waldman, J. Mol. Graph. Model**, 2003**, *21*, 289-307.
38. A. K. Ghose, G. M. Crippen, J. Comput. Chem., **1986**, *7*, 565-577.
39. A. K. Ghose, G. M. Crippen, J. Chem. Inf. Comput. Sci., **1987**, *27*, 21-35.
40. V. N. Viswanadhan, A. K. Ghose, G. R. Revankar, R. K. Robins, J. Chem. Inf. Comput. Sci., **1989**, *29*, 163-172.
41. A. K. Ghose, A. Pritchett, G. M. Crippen, J. Comput. Chem., **1988**, *9*, 80-90.
42. A. K. Ghose, V. N. Viswanadhan, J. J. Wendoloski, J. Phys. Chem. A., **1998**, *102*, 3762-3772.
43. C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, Adv. Drug Del. Rev., **1997**, *23*, 3-25.
44. C. A. Lipinski, F. Lombardo, B.W. Dominy, P. J. Feeney, Adv. Drug Del. Rev., **2001**, *46*, 3-26.
45. T. I. Oprea, A. M. Davis, S. J. Teague, P. D. Leeson, J. Chem. Inf. Comput. Sci., **2001**, *41*, 1308-1315.