Insights from the predicted interactions of plant derived compounds to the gluco-corticoid receptor as an alternative to dexa-methasone

Rajeev Sarmah

Centre for Bioinformatics Studies, Dibrugarh University, Dibrugarh 786004, Assam India; Rajeev Sarmah - E mail: akendrarajeev@gmail.com

Abstract:
Dexamethasone (DEX) an anti-inflamatory 9-fluoro-glucocorticoid, activates the cytosolic glucocorticoid receptor (GR) binding to its Ligand Binding Domain (LBD). The GR-ligand complex then translocates to the nucleus and binds to the Glucocorticoid Response Element (GRE) resulting up-regulation of target gene expression of anti-inflamatory proteins. DEX is one of the most effective ligand for GR activation but comply to side effects. Therefore, alternative for DEX – plant metabolites of *Calotropis sp* and *Swertia chirata* were screened using docking appraoch. These plants compounds were selected because; parts of these plants are widely used againsts inflamation, allergy, asthma etc. Three metabolites of *Swertia chirata* namely Gentianine (GENT), Xanthone (XANT) and Swerchirin (SWER) are found to be occupying the same binding pocket in the LBD of the GR (PDB ID 1M2Z). The binding affinity as reflected by binding energies of GENT-1M2Z, XANT-1M2Z and SWER-1M2Z are -5.6, -6.7 and -6.7, and all the output parameter of the respective compounds positively correlates with that of DEX-1M2Z with r = 0.9, 0.6 and 0.6 respectively indicating similar GR activation function. Visualization analysis of the models clearly indicates that GENT and SWER may be GR activators. Rest of the compounds mostly docked onto the surface of the receptor molecule.

Keywords: Natural Compounds, Docking, Gucocoticoid Receptor, Anti-inflamation

Background:

A broad spectrum of physiologic function essentially important for life is regulated by Glucocorticoids (GCs). The GCs play an important role in maintaining of the basal and stress related homeostasis [1-2]. GCs such as DEX bind to the cytosolic GRs, which gets activated by ligand binding. After a ligand binds to a corresponding receptor, the newly formed GC-GR complex, gets translocated into the cell nucleus, where it binds to Glucocorticoid Response Element (GRE) in the promoter region of the target genes resulting upregulation of anti-inflamatory protein expression – transactivation. The anti-inflamatory GRs lead to enhanced synthesis of the proteins collectively known as lipocortins, which inhibit phospholipase A2, leading to decreased eicasonoid synthesis [3]. GRs are part of the feedback mechanism in the immune system that turns immune activity or inflamation down. These are therefore, used as medicine to treat diseases caused by an overactive immune system such as allergies, asthma, autoimmune diseases and sepsis. DEX is one of the potential GCs used for the treatment of inflammatory and autoimmune conditions such as rheumatoid arthritis [4] and asthma [5]. DEX is also prescribed by dentists in small amounts before and/or after some forms of dental surgery [6], often it is administered before use of antibiotics in cases of bacterial meningitis, to reduce the inflammatory response of the body to the bacteria killed by the antibiotics [7].

Although GCs have potentially diverse regulatory effects but induces many side effects upon chronic use "Patients receiving

chronic steroids have an increased susceptibility to many different types of infections. The risk of infection is related to the dose of steroid and the duration of therapy **[8]**. The chronic use of steriods therefore, may also invite and increase the chances of incidence of most of the pathogenic organisms.

In India, since time immemorial use of indigenous plants are prevelant against many disease and some are also used against inflamation - ayurvedic medicine or anthropogonial treatment. Traditionally, the plants such as *Calotropis* sp. and *Swertia chirata* are applicable against inflamation, the root of *Calotropis gigantea* is used in treatment of inflamatory response because of leprosy, asthma, bronchitis, and expectorant. Root of *Calotropis gigantia* contains α-amyrin, β-amyrin, taraxasterol, β-sitosterol, stigmasterol **[9, 10]**, α-and β-amyrin are reported to possess anti-lipoxygenase activity **[11]**. The compunds of *Calotropis gigantea* are reported as free radical scavenger **[12, 13]**; having procoagulation **[14]** and wound healing activity **[15]**; also are antidiarrheal **[16]**, anticonvulsant **[17, 18]**, analgesic **[19]**, pregnancy interceptive **[20]**, anti-inflammatory **[21-24]**, hepatoprotective **[25, 13]** and Anti-diabetic **[26]**. *Swertia chirata* contain amarogentin a topoisomerase inhibitor revealed by Ray *et al.* (1996), amaroswerin Gastro-shielding reported by Niiho Y *et al.* (2005), gentianine Anti-inflammatory, anesthetic, antihistaminic reported by Song Zhen Yu *et al.* (1958), Geng Tao *et al.* (1959) and Kwak *et al.* (2005), swerchirin is an antimalarial compound, suggested by Arino *et al.* (1997), and swertiamarin is having analgesic property reported by Lei *et al.* (1982) **[27]**. The review suggests that the compounds of the *C. gigantia* and *S. Chirata* are having manifold medicinal role, however, reports on the molecular interactions that may take place for the respective physiologic functions are lacking. The present work therefore, is to simulate the interataction of the compounds of *C. gigantia* and *S. chirata* with 1M2Z a crystallographic model of a GR – regulated for anti-inflamation by DEX so as to get a probable alternative to DEX and understand the insight of the interaction through docking simulation approach.

Methodology:
The information about ligand molecules for docking simulation Taraxterol (TARA), Beta-amyrin (BAMA), Alpha-amyrin (AAMA) Beta-sitosterol (SITO) and Stigmasterol (STIG) of *Calotropis sp.* and Amarogentin (AMAR), Amaroswerin (ASWE), Gentianine (GENT), Swerchirin (SWER), Swertiamarin (SAMA), Xanthone (XANT), Mangiferin (MANG) and Syringaresinol (SYRI) of *S. Chiraytia* were downloaded from PubChem chemical database of NCBI (http://pubchem.ncbi.nlm.nih.gov) [28]. The 3D coordinate file of the GR (1M2Z) was retrieved from the Protein Data Bank (PDB). The Docking simulation was carried out using AutoDock 4.0 suite **[29]**. Before docking, the protein structures file was cleaned, removing the H_2O molecules and HETATM, and then H-atoms were added to the target protein to attain correct ionization and tautomeric states of amino acid residues. Further, Gasteiger charges were added to the receptor molecule. The ligands to be docked were kept flexible, so as to explore number of torsional degrees of freedom in addition to the translational and rotational parameters. The rigid roots of each ligand were defined automatically; all rotatable dihedrals in the ligands were allowed to rotate freely. The required precalculated grid map was prepared covering the chain A of 1M2Z (1M2Z: A). To prepare, run and analyze the docking

simulation the GUI program AutoDockTools (ADT) was used. The Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers. During the docking process, a maximum of 10 conformers was considered for each compound. The population size was set to 150 and the individuals were initialized randomly. Maximum number of energy evaluation was set to 2500000, maximum number of generations 1000, maximum number of top individual the automatically survived set to 1, mutation rate of 0.02, crossover rate of 0.8, step sizes were 0.2 Å for translations, 5.0 for quaternions and 5.0 for torsions. Cluster tolerance 0.5Å, external grid energy 1000.0 max initial energy 0.0 max number of retries 100000 and 10 LGA runs were performed. All the AutoDock docking runs were performed in Intel Pentium vostro 1510 3.0 GHz of Dell with 1GB RAM. AutoDock 4.0 was compiled and run under Ubuntu 11.10 OS. Before docking of the compounds of *C. gigantea* and *S. chirata*, the 1M2Z chain was first docked using DEX ligand after removal of the existing bound ligand, so as to acertain whether the parameters used for study are satifactory. The ADME toxicity and other descriptors of the compounds were explored using online softwares Molinspiration, Osiris and FAFDrug2 **[30-32]**.

Discussion:
The Ligands
The structure of the compounds of *Calotropis sp.* and *S. chirata* were downloaded from the PubChem compound database of NCBI are shown in **(Figure 1)**. The descriptors of these compounds shown in **Table 1 (See supplementary material)** only the result of FAFDrug2 has been shown since the entire three properties explorer computed similar scores for the descriptors. Except the compunds amarogentin and amaroswerin rest of the compounds have molecular weight < 500. Each of the compounds of *Calotropis* sp. are violating 1 lipinski rule, where as amarogentin, amaroswerin and mangiferin of *S. Chirata* are violating 3, 3 and 2 respectively. The logP value of all compounds of *Calotropis* sp. are high determining low hydrophlicities and therefore accounting to poor solubility whereas the compounds of *S. Chirata* are indicated to be readily soluble. The Topological Polar Surface Area (TPSA) of all compounds reveals as good human intestinal absorbant, Caco-2 monolayers permeable, and blood-brain barrier penetrator. Among the ligands the syringaresinol of *S. Chiraytia* achieved a better drug score of 0.7 table1, but it may be moderately irritant. The bioavailabilities of all of these compounds are reported to be good and are acceptable candidate drug reported by FAF-drug2, **Table 1 (See supplementary material)**. The comparative analysis of the values for molecular descriptor of DEX to all others reveals that the compounds of *S. chirata* have higher positive correlation of r > 9, **Table 1**.

The GR (1M2Z)
The 3D coordinate file of the crystal structure of the glucocorticoid receptor (GR) was downloaded from PDB [PDB ID: 1M2Z]. This receptor has dimer interface, consisting of a ligand binding pocket composed of residues from six helixes, an N-terminal activation function (AF') domain, a central DNA binding domain and a C-terminal ligand binding domain (LBD) with an ligand dependent activation function (AF) helix **(Figure 2A) [33]**. The existing bound ligand occupies 65% volume of the pocket **[33]**. The binding affinity is because of the hydrophilic

and hydrophobic interaction and hydrogen bonding between the ligand and the receptor molecule. From the downloaded PDB coordinate file of 1M2Z, HETATM or the ligand DEX was removed from the receptor to vacate the binding pocket and then DEX was allowed to dock into one of dimer made of chain A [1M2Z:A]. As expected the ligand docked into the same binding pocket of the receptor and shown similar hydrophilic and hydrophobic interactions explaining higher binding affinity equating to the existing crystellographic model **(Figure 2A)**.

However, slight deviation in polar contacts is also visualised **(Figure 2B)**, the comparative analysis of the docked outputs for all best conformers to that of DEX-1M2Z. A docked model reveals that XANT, GENT and SWER, occupied the same binding pocket with binding properties positively correlating r > 6 **Table 2 (see supplementary material)**, rest of the compounds although docked but on to the surface of the receptor molecule **Table 2**.
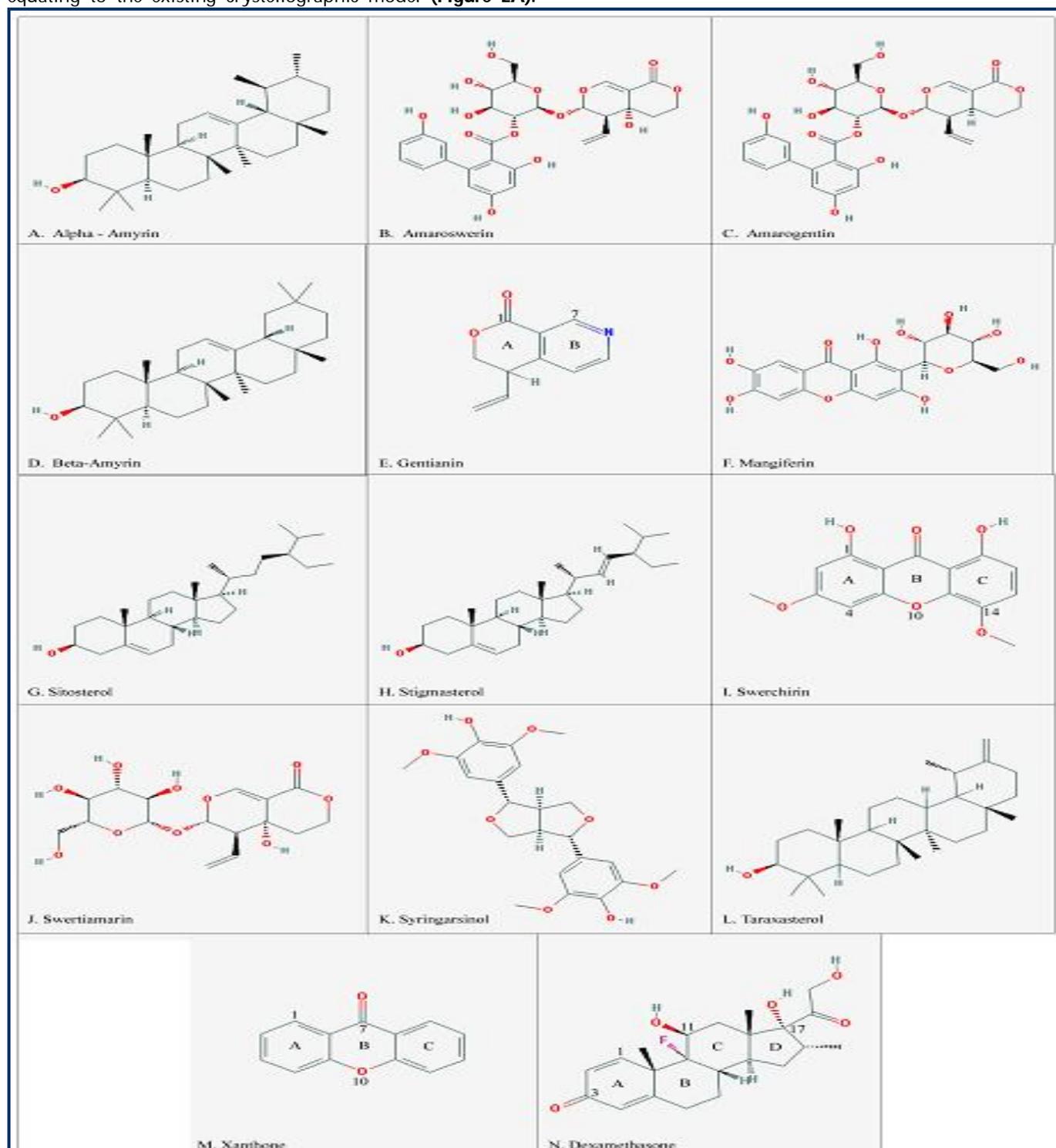
Figure 1: The characters in caps showing the ring codes and number showing the position of C in the molecule. These marking are shown only in those molecules that bind to the LBD pocket.

965

Docking of DEX into GR (1M2Z: A)

Docking simulation of DEX into GR (1M2Z: A) resulted in seven cluster of coformers result of the best conformer is shown in **Table 2**. The DEX docked into the same active site, described in the experimentally determined model 1M2Z, orienting with its A ring towards β strands and 1 and 2 helix and its D ring towards the AF helix, an extensive hydrophobic and hydrophilic interaction is seen between the ligand and the receptor, however, a slight deflection towards the β strand because of the 4 rotatable bonds of the ligand molecule is visualized **(Figure 2B)**. In the crystal complex the complex stability is attributed to the hydrogen bonds formed with the A ring carbonyl to the guanidium of R611 and to the γ-amid to

Q570, the side chain of N564 forms H-bonds to C ring 11-hydroxyl and 24-hydroxyl, 21 hydroxyl and 22-carbonyl forms H-bonds with residues Q624 and T739 respectively besides the hydrophilic and hydrophobic interactions. While in the DEX-1M2Z:A the insilico docked complex shows probable polar contacts of A ring carbonyl to the guanidium of R611, the g-amid of Q570 or with the carbonyl of M604, besides the H-bond between the side chain of N564 to C ring 11-hydroxyl, the hydroxyl may also establish polar contact with the side chain of L563, 21 hydroxyl and 24 hydroxyl may form H-bonds with residues Q624 and T739 respectively, 22-carbonyl of the C ring do not show any polar contact **(Figure 2B)**.
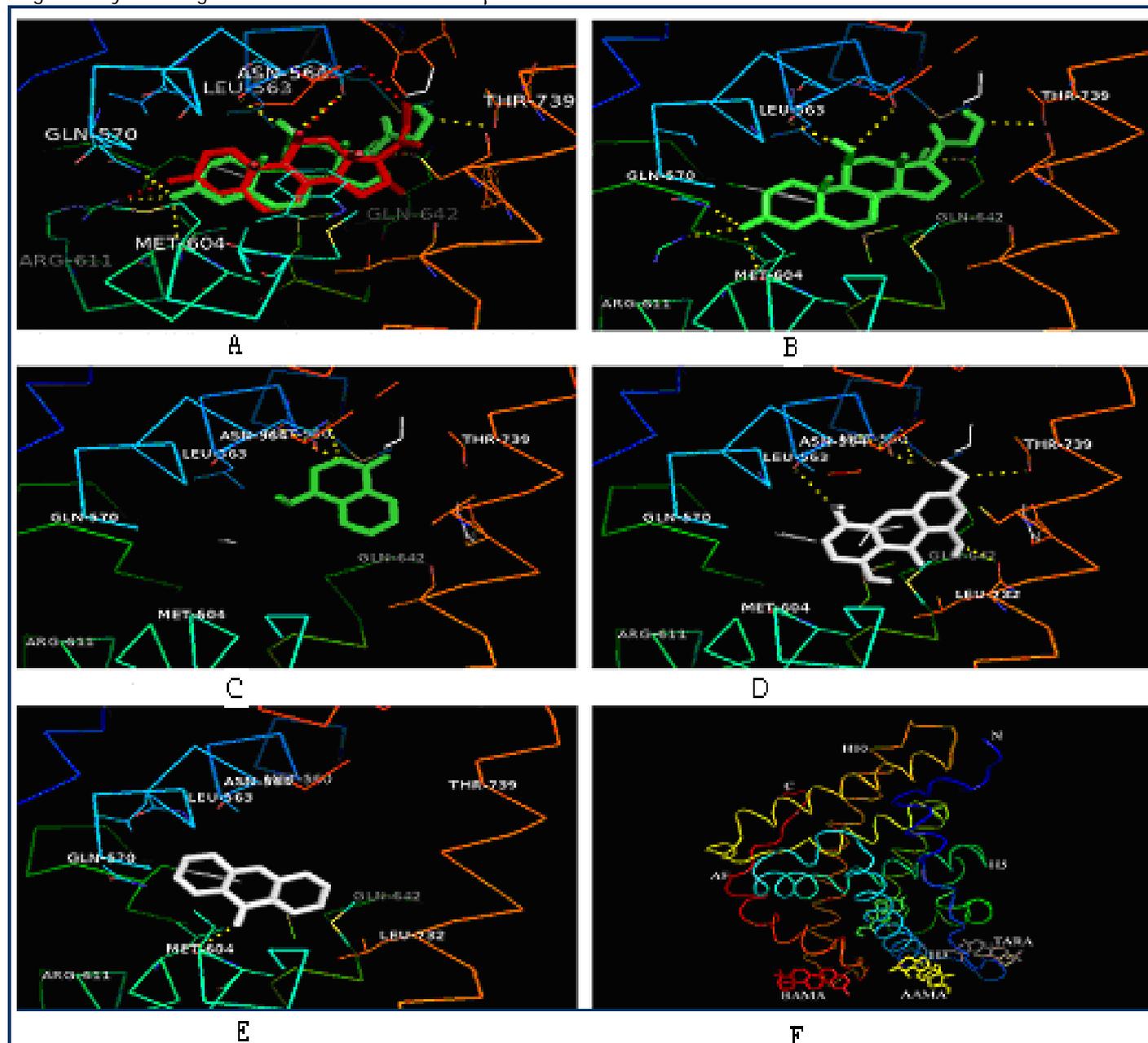
Figure 2: (A) Cartoon showing the binding pocket occupied by DEX in experimental model (red-colored ligand) and docked (green colored) using AutoDock4; **(B)** artoon showing the probable polar contacts of DEX in the pocket of IM2Z; **(C)** cartoon showing GENT docking into the binding pocket; **(D)** cartoon showing SWER docking into the binding pocket; **(E)** Cartoon showing XANT docking into the binding pocket; **(F)** Cartoon the helixes of the 1M2Z and the surface docked BAMA (red), AAMA (yellow) and TARA (wheat) colored.

Docking of GENT into GR (1M2Z: A)

GENT a compound of *S. chirata* docked into the same pocket as DEX with binding energy -5.56 **Table 2 (See supplementary material)**. Comparatively molecular weight and tpsa of GENT is much lesser than DEX. Approximately 15-20% of the volume is occupied by the ligand effectively with polar contacts of 2-Oxygen and the side chain of M560 and Q564 moreover; hydrophylic and hydrophobic interaction of the surrounding residues may be attributed for the stability of the complex. Although it occupy a small volume in the binding pocket the ligand stays more closely towards the AF helix which may be an important consequence leading to the activation of GR **(Figure 2C)**.

Docking of SWER into GR (1M2Z: A)

The model of SWER-1M2Z: A reveals that the ligand docked into the LBD of the receptor occupying almost 40-50% of the volume of the pocket with a binding energy of -6.74 **Table 2 (see supplementary material)**. Visual analysis of the model shows that the ligand is oriented with its C ring towards the AF helix and A ring towards tha β strand **(Figure 2D)**. The stability of the complex is explainable because of the extensive hydrophilic and hydrophobic interaction of the residues in proximity to SWER. Further stabilization, is attributed to the probable polar contacts established by 14-carbonyl of C ring with the B-carbonyl of L563, 3 and 1- carbonyl of A ring with the side chain of T739 and L732.

Docking of XANT into GR (1M2Z: A)

In this dock model the ligand is oriented with its C ring facing towards the AF helix and A ring towards tha B strand. The occupy a deeper region of the cavity towards the β- strand. Besides the hydrophilic and hydrophobic interactions of the residues in proximity, the complex stabilization can be attributed to the polar contact of 7-carbonyl of B-ring with the β-caronyl of M604 **(Figure 2E)**. Visualization of the interaction at vendarwaals (vdw) scaling factor of 1.4 Å radius the GENT-1M2Z: A and SWER-1M2Z: A seems to be more stable than XANT-1M2Z: A complex. The GENT and SWER makes a weak contact with AF helix (L753) at vdw scaling factor 1.4 Å and the loop preceding the AF-helix (I747 and F749), therefore, are suspected to stablize the AF-helix in the active conformation and may serve as a molecular basis for ligand -dependent activation of GR, on the other hand being deep inside the binding pocket towards the β-strand chances of XANT as a GR activator is reduced **(Figure 2E)**.

Docking of other compounds into GR (1M2Z: A)

Rest of the compounds under study although docked, with better binding energy such as BAMA, AAMA, and TARA, are -7.71, 7.68, and -7.64 respectively, but far away from the LBD. The docked optput poorly correlates with DEX-1M2Z: A complex. Visualization of the docked the complexes reveals that most of ligands docked on the surface of the 1M2Z: A, below the AF helix as in the case of BAMA **(Figure 2F)**, or below the H3 helix as in the cases of AAMA and TARA. Among all of these compounds BAMA shows a better binding energy, however, the activation of the GR may not be fruitful because if occupancy of the binding pocket is essential. The docking simulation as a whole shows that the compounds GENT, SWER are the better compound for the activation compound of GR and may be alternative to DEX.

Conclusion:

Herbal medicines remain the major source of health care for the world since time immemorial. The plant kingdom represents a rich source of organic compound inspite of advances in modern system of medicine, there are several areas like tropical disease, herpes, AIDS, cancer, bronchial asthma etc., which still remain a challenge to present day drug therapy. Therefore, the search of natural product alternative to DEX by docking approach concludes that the compounds such as GENT and SWER may remain effective against inflamation, where GENT was already been reported as an anti-inflamatory by Song Zhen Yu et al., (1958) **[32]**. Here, the molecular basis of the GENT action repoted theoriticaly by docking approach and together with GENT this docking simulation also reveals that the SWER of *S. chirata* may be another anti-inflamatory ligand. As *S. chirata* is commonly used in Assam for the releaf of cough or asthmetic trouble and the binding of three compounds into the active site of the GR receptor with better binding energies, hence explain the use of the plant as medicine against inflamation is one of the correct anthropology.

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Supplementary material:

Table 1: Showing molecular descriptors of the compounds.

ID	MW	logP	logSW	tPSA	RotB	RigB	HBD	HBA	HB	ratioH/C	L_V	Sol(mg/l)	Oral_bio	S_C	r	DS
TARA	426.72	9.13	-8.24	20.23	0	27	1	1	2	0.03	1	112.88	Good	10	0.19828	0.15
BAMA	426.72	9315	-8.25	20.23	0	26	1	1	2	0.03	1	111.47	Good	8	0.19557	0.15
AAMA	426.72	9.01	-8.16	20.23	0	26	1	1	2	0.03	1	121.75	Good	10	0.21840	0.14
SITO	414.71	9.34	-7.90	20.23	6	20	1	1	2	0.03	1	153.84	Good	9	0.29782	0.11
STIG	412.69	8.56	-7.46	20.23	5	21	1	1	2	0.03	1	237.22	Good	9	0.46402	0.19
AMAR	586.54	2.42	-4.60	201.67	8	32	6	13	19	0.45	3	5916.57	Good	8	0.99752	0.29
ASWE	602.54	1.34	-4.01	221.90	8	32	7	14	21	0.48	3	10886.92	Good	8	0.99965	0.31
GENT	175.18	1.34	-1.91	39.19	1	13	0	3	3	0.30	0	26041.15	Good	1	0.99971	0.49
SWER	288.25	2.75	-3.54	89.13	2	17	2	6	8	0.40	0	8349.76	Good	0	0.99999	0.46
SAMA	374.34	-2.00	-0.64	155.14	4	19	5	10	15	0.63	0	197997.99	Good	8	0.99959	0.45
XANT	196.20	2.96	-3.37	30.21	0	17	0	2	2	0.15	0	6735.95	Good	0	0.99999	0.40
MANG	422.34	-0.37	-2.31	201.28	2	23	8	11	19	0.58	2	41953.87	Good	5	0.99979	0.41
SYRI	418.44	2.23	-3.60	95.84	6	21	2	8	10	0.36	0	11412.91	Good	4	0.99998	0.70

r: correlation coeficient; DS: druglikeliness score; L_V: lipinski violation; S_C: stereo centre; HB hidrogen bonds; HBD: H-bond donor; HBA: H-bond acceptor; RigB: rigid bond; RotB: rotatble bonds; tPSA: topological polar surface area.

Table 2: Showing the output of the docking simulation.

Docks	B_E	kI	I_E	In_E	T_E	U_EE	R_RMS	r
DEX-1M2Z:A	-9.59	93.13	-11.08	-0.53	1.49	-0.53	31.71	1
GENT-1M2Z:A	-5.56	84.29	-5.86	-0.11	0.3	-0.11	31.85	0.9983560925
SYRI-1M2Z:A	-4.43	568.32	-6.81	-1.83	2.39	-1.83	35.17	0.948051382
AMAR-1M2Z:A	-4.16	886.58	-8.34	-4.31	4.18	-4.31	37.4	0.9413765806
ASWE-1M2Z:A	-2.24	22.8	-6.71	-5.55	4.47	-5.55	38.71	0.7064984515
STIG-1M2Z:A	-6.57	15.29	-8.36	-0.84	1.79	-0.84	36.93	0.6177651501
XANT-1M2Z:A	-6.69	12.51	-6.69	0	0	0	31.37	0.609280936
SWER-1M2Z:A	-6.74	11.55	-7.93	-1.28	1.19	-1.28	31.33	0.5956019478
SITO-1M2Z:A	-6.76	11.11	-8.85	-1.65	2.09	-1.65	37.17	0.5409651304
MANG-1M2Z:A	-3.24	4.21	-6.22	-2.87	2.98	-2.87	30.89	0.3996340882
TARA-1M2Z:A	-7.64	2.49	-7.94	0.05	0.3	0.05	35.96	0.3544876363
AAMA-1M2Z:A	-7.68	2.33	-7.98	0.05	0.3	0.05	36.67	0.3486245377
BAMA-1M2Z:A	-7.71	2.22	-8.01	0.05	0.3	0.05	36.45	0.3470888249
SMAR-1M2Z:A	-3.91	1.36	-6.59	-4.88	2.68	-4.88	29.72	0.346698798

B_E: binding energy; I_E: intermolecular energy; In_E: internal energy; T_E: torsional energy; U_EE: unbound extended energy; R_RMS: reference RMS; r: correllation coefficient