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Original Article

Virtual drug screening for p65/rela subunit of nf-κb: Promising repurposable drugs in the treatment of stress-based diseases

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ABSTRACT

Background and Aims: Although NF-κB is composed of five subunits, RelA receives much more attention due to fact that its expression level is regulated under various stress conditions, such as exposure to radiation, reactive oxygen species (ROS), hypoxia, pathogens, and inflammatory cytokines, as well as regulating many inflammatory, proliferation, and apoptosis genes. To date, many pieces of evidence have demonstrated that RelA plays a significant role in in the prognosis of various proliferative and inflammatory diseases. Therefore, the design of novel inhibitors and the discovery of repurposable drugs are considered promising approaches in the treatment of RelA-based diseases.

Methods: A drug library including a total of 12,111 ligands has been screened for the RelA subunit of NF-κB. The sufficiency of the study's strategy has been revealed by analysis of commercially available inhibitors and re-docking applications.

Results: Findings demonstrate that ZINC000096928979 (Deleobuvir), ZINC000012503187 (Conivaptan), and ZINC000003974230 ligands have the highest binding affinity to RelA. Furthermore, many ligands with structural similarities to Valstar, Ergotamine drugs and Benzo[a]pyrene-7,8-Diol metabolite have been discovered.

Conclusion: While the ligands with the highest binding affinities could be repurposed in the treatment of RelA-based diseases, the structures of the ligands exhibiting similarity with Valstar, Ergotamine, and Benzo[a]pyrene-7, 8-D may be used as a scaffold in structure-based drug design studies. The stability of the interactions between the ligands and the receptor should be analyzed with further Molecular Dynamics Simulations (MD) studies and the possible ligands should be investigated by both in vitro and in vivo applications.

Keywords: RelA (p65), NF-κB, Virtual Drug Screening, Molecular Docking, Drug Repurposing

INTRODUCTION

Nuclear factor-κB (NF-κB) is one of the main transcription factors due to its regulatory activity on many significant cellular pathways such as apoptosis (Bernal-Mizrachi, Lovly, & Ratner, 2006), proliferation (Wan & Lenardo, 2010), differentiation (Kaltschmidt, Greiner, & Kaltschmidt, 2021), and inflammation (Liu, Zhang, Joo, & Sun, 2017). NF-κB is composed of five subunits: NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), RelB, and c-Rel (Perkins & Gilmore, 2006). Transcription of target genes requires nuclear translocation of NF-κB subunits through canonical and noncanonical signaling pathways (Sun, 2011; Zarnegar, Yamazaki, He, & Cheng, 2008). While these proteins may form several homodimers and heterodimers, these forms have distinct signaling mechanisms for the expression of various genes (Ghosh, Wang, Huang, & Fusco, 2012). The most abundant form of NF-κB is observed as heterodimers of NF-κB1 and RelA and the phosphorylation of RelA plays a significant role over the activity of this heterodimer since it provides chemical stability and causes conformational changes for protein-protein interactions (Chuang, Rehan, & Khorram, 2020; Darwish, Abo-Youssef, Messiha, Abo-Saif, & Abdel-Bakky, 2021).

While the activation of RelA is observed in endothelial cells (Bijli, Fazal, & Rahman, 2012), macrophages (Dorrington & Fraser, 2019), and smooth muscle cells (Zhang et al., 2010), various stresses such as radiation (Kim et al., 2004), reactive oxygen species (ROS) (Morgan & Liu, 2010), hypoxia (Choi et al., 2019), the existence of pathogens within the host body (Rahman & McFadden, 2011), and recognition of inflammatory cytokines (Ronin et al., 2019) enhance the expression level of RelA. Considering the activation of RelA by stresses, its expression regions and regulatory effect on proliferation, apoptosis

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and inflammatory genes, the connection between RelA and several proliferative diseases such as various cancer types (Zhang, Ma, Zhang, Zhang, & Hu, 2021), inflammatory diseases such as rheumatoid arthritis (Makarov, 2001) and inflammatory bowel diseases (Balta, 1998), and muscle tissue diseases such as multiple sclerosis (Zhou, 2020) have been reported in the literature. As such, inhibition of RelA shows promising potential in the treatments of related diseases (Giridharan & Srinivasan, 2018).

In this study, a molecular docking-based virtual drug screening targeting the RelA subunit of NF-κB was performed. Primarily, a drug library including 12,111 ligands composed of four distinct datasets; FDA-Approved Drugs (1,615 ligands), World-not-FDA Approved Drugs (4,288 ligands), Drugs in Clinical Trials (3,897 ligands), and Non-human Metabolites (2,311 ligands) was created and screened during the study. In addition, 16 commercially available inhibitors as well as the ligand found in the chemical structure of RelA (S-Adenosylmethionine (SAM)) were analyzed through the same experimental procedure for validation. Findings point that ZINC000096928979 (Deleobuvir), ZINC000012503187 (Conivaptan), and ZINC000003974230 ligands have the highest affinities in order to interact with RelA. In addition, many structurally similar ligands with Valstar, Ergotamine, and Benzo[a]pyrene-7,8-Diol ligands have binding affinity to RelA. Thus, results demonstrate that while the three best scored ligands might be considered as promising to be tried in the treatment of RelA based diseases, the structures of Valstar, Ergotamine, and Benzo[a]pyrene-7,8-Diol might be considered as scaffolding for further structural based drug design studies.

Materials and Methods

Receptor Preparation

The crystal structure of RelA subunit of NF-κB was retrieved from the Protein Data Bank (PDB) in .pdb format (PDB ID: 3QXY). The resolution, R-value (free), R-value (observed) parameters of the selected RelA subunit's were 2.09 Â, 0.229, and 0.173, respectively. Preparation of the receptor was carried out through the Dock Prep module of UCSF Chimera software version 1.16 by adding hydrogen atoms, partial charges and replacing the side chains with the Dunbrack 2010 rotamer library to remove the ligands, heteroatoms, and water. The prepared receptor was exported in .pdb format for further molecular docking studies (Pettersen et al., 2004).

Ligand Library Preparation

A drug library including 12,111 ligands was created by retrieving FDA-Approved Drugs (1,615 ligands), World-not-FDA Approved Drugs (4,288 ligands), Drugs in Clinical Trials (3,897 ligands), and Non-human Metabolites (2,311 ligands) datasets from the ZINC15 database. The ligands of the library were prepared through the energy minimization module of PyRx Virtual Screening Tool after importing the data separately (Dallakyan & Olson, 2015).

Molecular Based Drug Screening

Molecular docking based virtual drug screening of the prepared library was carried out with the AutoDock Vina package of PyRx Virtual Screening Tool by targetting the region interacting with S-Adenosylmethionine (SAM) inhibitor (Trott & Olson, 2011). For this purpose, the ligands were converted to .pdbqt format, and grid box parameters were defined as 20 x 20 x 20 as size, and x= 61.728, y= 7.720, z= 61.982 as coordinates. The data showing binding affinity, rmsd/ub, and rmsd/lb values of the ligands were exported in .csv format. The modes of the best scored ligands with 0 rmsd/ub, and 0 rmsd/lb values were selected, and the interactions between selected ligands with the receptor were analyzed in Biovia Discovery Studio Visualiser software.

Validation

A validation study was carried out by exporting the S-Adenosylmethionine (SAM) inhibitor found in chemical structure of RelA, following the same ligand preparation, and molecular docking procedures. The RMSD difference between SAM in the crystal structure and re-docked form was analyzed with DockRMSD web server produced by Zhang Lab (Bell & Zhang, 2019). As such, the SAM was exported from the retrieved pdb file, and both conformations were imported to the server in mol2. format. A total of 27 atoms were aligned by server and RMSD value pointing the sufficiency of the study was analyzed. In addition, a novel Inhibitors Library composed of 16 commercially available inhibitors of RelA, Licochalcone D, Stachydrine, Sauchinone, Neferine, SC75741, Dihydroartemisinin, 5-Aminosalicylic Acid, Neochlorogenic Acid, Mangiferin, Morusin, Tectochrysin, Sulfasalazine, Tomatitine, Maslinic Acid, Vanillic Acid, and (-)-DHMEQ (Compound CIDs: 10473311, 115244, 11725801, 159654, 23661638, 3000518, 4075, 5280633, 5281647, 5281671, 5281954, 5339, 65576, 73659, 8468, 9881652, respectively) was created by retrieving the ligands from PubChem database. The molecular docking procedure was repeated with this library in order to analyze the efficiencies of the inhibitors.

ADME and Toxicity Properties

Absorption, Distribution, Metabolism, and Excretion (ADME) and toxicity properties of two of the best scored ligands (ZINC000096928979 (Deleobuvir) and ZINC000003974230) with the three best scored inhibitors were analyzed with both the swissADME server (Daina, Michielin, & Zoete, 2017) and OSIRIS Property Explorer tool (Sander, 2022). Therefore, the

ligands' physicochemical, solubility, lipophilicity, pharmacokinetics properties, and toxicity profiles were studied. Since it had been tested and approved by FDA previously, ADME and toxicity analyses were not required for ZINC000012503187 (Conivaptan).

Results and Discussion

Virtual drug screening for the RelA subunit of NF-κB was carried out in order to reveal possible repurposable drugs. Therefore, a drug library consisting of FDA-Approved Drugs, World-not-FDA Approved Drugs, Drugs in Clinical Trials, and Non-human Metabolites datasets were created and a total of 12,111 ligands were docked to the inhibitor binding region of RelA. The results including the binding affinities and the datasets of the 20 best scored ligands as well as the interacting amino acid residues of the receptor with the related ligands are listed in Table 1.

In order to validate the molecular docking strategy, the S-Adenosylmethionine (SAM) inhibitor found in the chemical structure of RelA was exported as a separate file and was re-docked to the same region of the receptor. The re-docked SAM's binding affinity was recorded as -8.8 kcal/mol. Interactions between ligand and receptor in both the SAM re-docking study and the SAM in crystal structure of .pdb file were analyzed in Biovia Discovery Studio software (Figure 1). Accordingly, SAM in crystal structure interacts with ALA 73, TYR 75, TYR 223, ASN 251, HIS 252, TYR 297 residues through conventional hydrogen bonds, TYR 285 and EDO 477 through carbon-hydrogen bonds, VAL 72 through pi-sigma interaction, PHE 299 through pi-pi stacked interaction, ALA 73 through pi-alkyl interaction and the water molecules through water hydrogen bonds. Besides, re-docked SAM interacts with TYR 75, LEU 146, TRP 147, TYR 223, HIS 252, TYR 285 residues through conventional hydrogen bonds, ASN 251 through carbon-hydrogen bonds, ARG 68 through pi-cation interaction, PRO 148 through pi-alkyl interaction, and LEU 146 through unfavorable acceptor-acceptor interaction. Since water molecules had been removed during protein preparation, possible interactions with the ligands could not be analyzed. The RMSD difference between SAM in crystal structure and re-docked form were measured as 1.126 by DockRMSD web server of Zhang Lab. Observing common amino acids, similar interactions, and close RMSD values between re-docked SAM and SAM in retrieved file proves the sufficiency of study's strategy.

In addition, the Inhibitors Library composed of 16 commercially available inhibitors was created and docked to RelA to reveal the binding affinities and the common amino acids that are interacted with the inhibitors. The binding affinities of the Inhibitor Library including re-docked SAM and the interacting amino acids are listed in Table 2. Accordingly, three inhibitors, which are Morusin, SC75741, and Sauchinone have exhibited

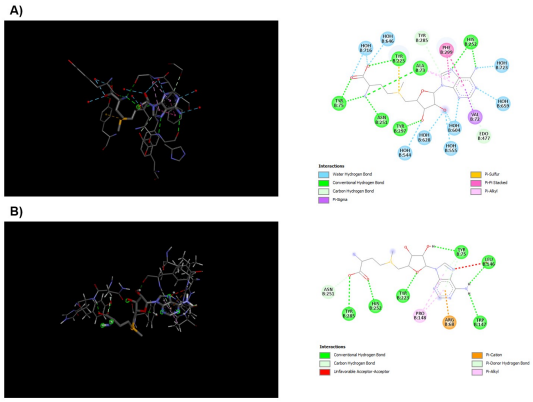


Figure 1. RelA interactions with A) S-Adenosylmethionine (SAM) in crystal structure and B) re-docked S-Adenosylmethionine (SAM).

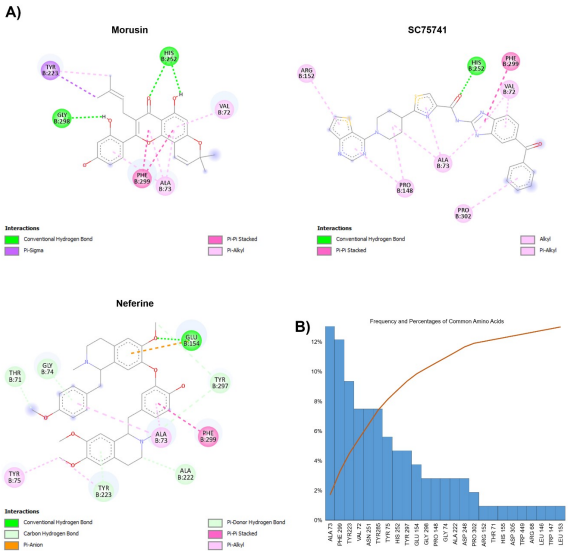


Figure 2. A) RelA interactions with the best scored inhibitors which are Morusin, SC75741, and Neferine, B) Frequency and percentages of common interacting amino acids.

high binding affinities as -10.9 kcal/mol, -10.6 kcal/mol, and -10.2 kcal/mol, respectively. Furthermore, Morusin interacts with HIS 252 and GLY 298 through conventional hydrogen bonds, VAL 72, ALA 73, TYR 223, and PHE 299 through pi-alkyl interactions, TYR 223 through pi-sigma interaction, PHE 299 through pi-pi stacked interactions, SC75741 interacts with HIS 252 through conventional hydrogen bonds, VAL 72, ALA 73, PRO 148, ARG 152, and PRO 302 through alkyl interactions; PHE 299 through pi-pi stacked interaction, VAL 72 and ALA 73; and PRO 148 through pi-alkyl interactions. Sauchinone interacts with GLU 154 through conventional hydrogen bonds, THR 71, ALA 222, and TYR 297 through carbon-hydrogen bonds, GLY 74 and TYR 223 through pi-donor hydrogen bonds, ALA 73, TYR 75, and TYR 223 through pi-alkyl interactions, PHE 299 through pi-pi stacked interaction, and

Table 1. Best Scored 20 Ligands' binding affinities, datasets, and the amino acid residues that interacted with.

30 Best Scored			
Ligand Name	**Score (kcal/mol)**	**Dataset**	**Receptor Residues Interacting with Ligands**
ZINC000096928979	-13.7	Drugs in Clinical Trials	VAL 72, ALA 73, TYR 75, TRP 147, TYR 223, ASP 248, LEU 250, ASN 251, PHE 299, TRP 449
ZINC000012503187	-13.0	FDA-Approved Drugs	ARG 68, ALA 73, TYR 75, LEU 146, PRO 148, GLU 154, ALA 222, TYR 223, PHE 289
ZINC000003974230	-12.9	Drugs in Clinical Trials	VAL 72, ALA 73, PRO 148, GLU 154, TYR 223, TYR 285, PHE 299
ZINC000095618662	-12.8	World-not-FDA Approved Drugs	ARG 68, VAL 72, ALA 73, TYR 75, PRO 148, TYR 223, ASN 251, HIS 252, TYR 285, TYR 297, PHE 299, VAL 300, PRO 302
ZINC000003922429	-12.3	Drugs in Clinical Trials	VAL 72, ALA 73, TYR 223, ASP 248, ASN 251, HIS 252, PHE 299
ZINC000004214612	-12.3	World-not-FDA Approved Drugs	ARG 68, VAL 72, ALA 73, TYR 75, LEU 146, TRP147, PRO 148, GLU 149, LEU 153, TYR 223, ILE 249, HIS 252, TYR 285, PHE 299, VAL 300, TRP 449
ZINC000004215812	-12.3	World-not-FDA Approved Drugs	ARG 68, VAL 72, ALA 73, TYR 75, GLU 154, TYR 223, PHE 299
ZINC000011616852	-12.3	FDA-Approved Drugs	ARG 68, VAL 72, ALA 73, GLY 74, PRO 148, GLU 149, ALA 222, TYR 223, PHE 225, ASN 251, HIS 252, TYR 285, TYR 297, PHE 299, VAL 300, PRO 302, TRP 449
ZINC000100016063	-12.3	Drugs in Clinical Trials	VAL 72, ALA 73, ALA 222, TYR 223, TYR 285, TYR 297, PHE 299
ZINC000043204146	-12.2	Drugs in Clinical Trials	VAL 72, ALA 73, TYR 75, GLU 149, ARG 152, GLU 154, TYR 223, HIS 252, TYR 285, PHE 299, GLU 301, PRO 302
ZINC000052955754	-12.2	FDA-Approved Drugs	ARG 68, VAL 72, ALA 73, GLY 74, TYR 75, PRO 148, TYR 223, PHE 299, VAL 300
ZINC000003924139	-12.1	Drugs in Clinical Trials	ARG 68, VAL 72, ALA 73, TYR 75, LEU 146, PRO 148, TRP 147, TYR 223, GLY 298, PHE 299
ZINC000003926844	-12.1	Drugs in Clinical Trials	VAL 72, ALA 73, TYR 75, PRO 148, ARG 152, LEU 153, GLU 154, ALA 222, TYR 223, HIS 252, TYR 285, PHE 299
ZINC000003978005	-12.1	FDA-Approved Drugs	ARG 68, VAL 72, ALA 73, TYR 75, PRO 148, ILE 249, PHE 299
ZINC000003985678	-12.1	Drugs in Clinical Trials	VAL 72, ALA 73, TYR 75, LEU 146, LEU 153, HIS 155, ALA 222, ASP 248, ILE 249, ASN 251, HIS 252, LEU 253, TYR 285, PHE 299
ZINC000006717791	-12.1	Drugs in Clinical Trials	VAL 72, ALA 73, LEU 153, HIS 155, ALA 222, TYR 223, ASP 248, TYR 285, PHE 299
ZINC000030728718	-12.1	Non-human Metabolites	VAL 72, ALA 73, TYR 75, TYR 223, SER 224, ALA 247, ILE 249, ASN 283, TYR 297, PHE 299
ZINC000063933734	-12.1	Drugs in Clinical Trials	VAL 72, ALA 73, ASN 251, ALA 222, TYR 223, ASP 248, TYR 285, TYR 297, GLY 298, PHE 299, TRP 449
ZINC000072190224	-12.1	Drugs in Clinical Trials	VAL 72, ALA 73, TYR 75, LEU 146, TRP 147, PRO 148, LEU 153, GLU 154, ALA 222, TYR 223, PHE 225, ASN 251, HIS 252, TYR 285, ASP 248, PHE 299
ZINC000095618690	-12.1	World-not-FDA Approved Drugs	ALA 73, TYR 223, ASP 248, ILE 249, PHE 299

GLU 154 through pi-anion interaction (Figure 2-A). The frequency and percentages of common amino acids interacting with inhibitors are shown in Figure 2-B. Accordingly, common interacting amino acids are VAL 72, ALA 73, GLY 74, TYR 75, PRO148, GLU 154, ALA 222, TYR 223, ASP 248, ASN 251, HIS 252, TYR 285, TYR 297, GLY 298 and PHE 299.

Virtual drug screening findings demonstrate that the three best scored ligands, ZINC000096928979 (Deleobuvir), ZINC000012503187 (Conivaptan), and ZINC000003974230 have binding affinities of 13.7 kcal/mol, 13.0 kcal/mol, and 12.9 kcal/mol, respectively, and possess high potential to be used in the treatment of RelA based diseases. The interactions of these ligands with RelA protein are demonstrated in Figure 3. The analysis put forward that ZINC000096928979 (Deleobuvir), which is used in the treatment of Hepatitis C (HCV) through inhibiting the NS5B polymerase (Larrey et al., 2013), might create conventional hydrogen bonds with ASN 251, carbon-hydrogen bonds with TRP 147, ASP 248, and LEU 250, pi-donor interactions with TYR 223, pi-sigma interactions with TYR 75, pi-pi stacked and pi-pi T-shaped interactions with TRP 449, and PHE 299, alkyl interactions with TRP 449, and pi-alkyl interactions with VAL 72, ALA 73, and TYR 223 residues. ZINC000012503187 (Conivaptan) which is used in hypervolemic and euvolemic hyponatremia (Zeltser, Rosansky, Van Rensburg, Verbalis, & Smith, 2007) as Vasopressin receptor inhibitor (Ali, Raufi, Washington, & Ghali, 2007) creates conventional hydrogen bonds with LEU 146, and TYR 223, pi-cation and pi-anion interactions with ARG 68, and GLU 154, pi-donor hydrogen bonds with TYR 75, pi-pi stacked interactions with PHE 299, pi-alkyl interactions with ALA 73, TYR 75, PRO 148, ALA 222, and TYR 223 residues. The ZINC000003974230 ligand, whose unknown activity creates conventional hydrogen bonds with TYR 223, has unfavorable acceptor-acceptor interactions with TYR 285, pi-anion interactions with GLU 154, pi-pi stacked interactions with TYR 223, TYR 285, and PHE 299, alkyl and pi-alkyl interactions with VAL 72, ALA 73, PRO 148, and PHE 299 residues. Since the interacting amino acids and interaction types exhibit similarity with the inhibitors, HCV inhibitor ZINC000096928979 (Deleobuvir), Vasoppressin receptor inhibitor ZINC000012503187 (Conivaptan), and ZINC000003974230 ligands are considered as repurposable in the treatment of stress based diseases progressed by RelA activation. Chemical structures of the best scored ligands and the inhibitors are shown in Figure 4.

ADME and possible toxicity properties of the best scored ligands, which are ZINC000096928979 (Deleobuvir), and ZINC000003974230, were carried out with the OSIRIS Property Explorer tool and swissADME server. In order to compare the potential of these ligands, the three best scored inhibitors were analyzed by the same strategy as well (Table 3). Since ZINC000012503187 (Conivaptan) had been approved by the FDA, it does not require analysis for ADME and toxicity properties. Findings demonstrate that ZINC000096928979

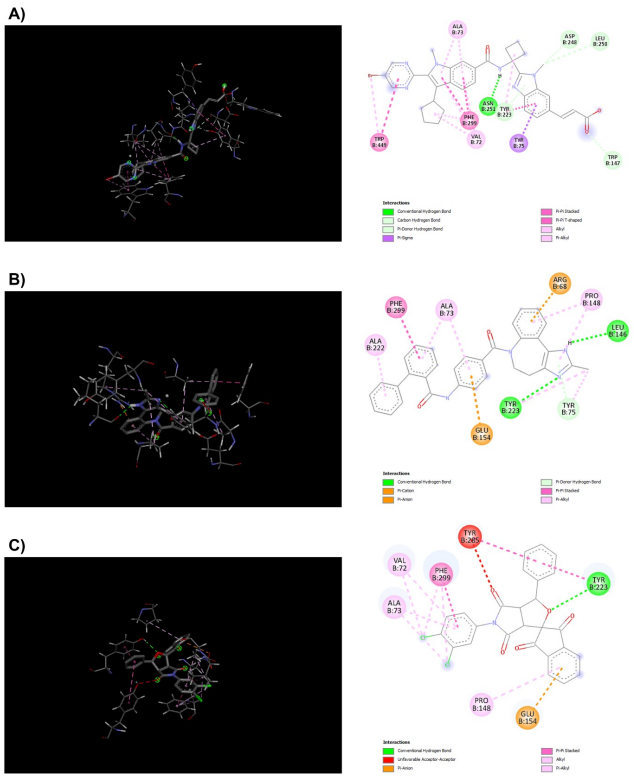


Figure 3. RelA interactions with the best scored ligands; A) ZINC000096928979 (Deleobuvir), B) ZINC000012503187 (Conivaptan), and C) ZINC000003974230.

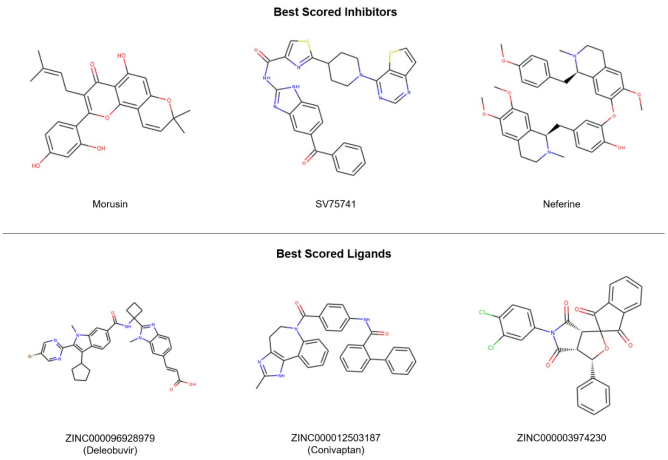


Figure 4. Chemical structures of the three best scored inhibitors and ligands from virtual screening.

(Deleobuvir) is poorly soluble, has low gastrointestinal (GI) absorption, no CYP isoform inhibition activity except CYP2C19 and CYP2D6, and no toxicity potential. ZINC000003974230 is moderately soluble, has high GI absorption, no CYP isoform inhibition activity except CYP2C19 and CYP2C9, and possible mutagenicity and reproductive effects. It has been demonstrated

Table 2. Inhibitor Library results including re-docked SAM.

Inhibitors Including Re-docked Benzamidine		
Ligand Name	**Binding Affinity (kcal/mol)**	**Receptor Residues Interacting with Ligand**
Morusin	-10.9	VAL 72, ALA 73, TYR 223, HIS 252, GLY 298, PHE 299
SC75741	-10.6	VAL 72, ALA 73, PRO 148, ARG 152, HIS 252, PHE 299, PRO 302
Neferine	-10.2	THR 71, ALA 73, GLY 74, TYR 75, GLU 154, ALA 222, TYR 223, TYR 297, PHE 299
Sauchinon	-9.8	ALA 73, HIS 155, PHE 299
Mangiferin	-9.5	VAL 72, ALA 73, GLU 154, TYR 223, ASN 251, TYR 285, TYR 297, GLY 298, PHE 299
Sulfasalazine	-9.4	VAL 72, ALA 73, TYR 223, ASN 251, TYR 285, PHE 299, ASP 305
Licochalcone D	-9.2	VAL 72, ALA 73, GLY 74, TYR 75, PRO 148, TYR 223, ASP 248, HIS 252, TYR 285, PHE 299, TRP 449
Tectochrysin	-8.9	VAL 72, ALA 73, ASN 251, HIS 252, TYR 285, PHE 299
S-Adenosylmethionine (SAM)	-8.8	ARG 68, TYR 75, LEU 146, TRP 147, PRO 148, TYR 223, ASN 251, HIS 252, TYR 285
Tomatidine	-8.8	ALA 73, TYR 297, PHE 299, PRO 302
Maslinic Acid	-8.7	GLU 154, ALA 222, TYR 223, TYR 285
Neochlorogenic Acid	-8.6	VAL 72, ALA 73, GLY 74, TYR 75, ASP 248, ASN 251, PHE 299
Dihydroartemisinin	-8.2	ALA 73, PHE 299
(-)-DHMEQ	-8.1	VAL 72, ALA 73, LEU 153, GLU 154, ASN 251, PHE 299
Vanillic Acid	-5.8	ALA 73, ALA 222, TYR 223, TYR 285, TYR 297, GLY 298, PHE 299
5-Aminosalicylic Acid	-5.7	ALA 73, TYR 75, TYR 223, ASP 248, ASN 251
Stachydrine	-4.9	TYR 223, ASN 251, TYR 285, TYR 297

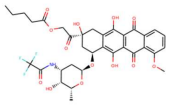
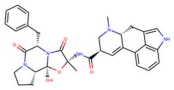
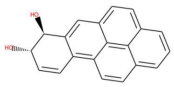
that these best scored ligands exhibit similarity with inhibitors about several parameters such as drug-scores, CYP inhibitory activities, and toxicity. In particular, since ZINC000096928979 (Deleobuvir) has no toxicity effects and a rather favorable drug-score, and ZINC000003974230 has low molecular weight compared to the inhibitors, these ligands and ZINC000012503187 (Conivaptan) might be considered as promising repurposable drugs.

One of the novel approaches to develop inhibitor molecules against target proteins is based on designing ligands by referencing the structures with potential. In order to reveal potential structure scaffolds, the 30 best scored ligands from four datasets were analyzed. Among 120 ligands, 16, 5, and 11 ligands share structurally similarity with ZINC000011616852 (Valstar), ZINC000052955754 (Ergotamine), and ZINC000002019693 (Benzo[a]pyrene-7,8-Diol), respectively. The scaffold structures and the ligands sharing similarity are listed in Table 4. The scaffold of Valstar composes five benzene rings and long Carbon (C) chain carrying Oxygen (O) and hydroxyl (OH) groups. In addition, Ergotamine structure composes five benzene rings connected with three cyclopropane via carbon atom. Lastly, Benzo[a]pyrene-7, an 8-Diol structure, composes five strictly connected benzene rings. While these scaffolds share common structures such as five benzene rings, accordingly, these structures might be considered as a template in structure based drug design studies for RelA inhibition.

Table 3. ADME and toxicity properties of the three best scored inhibitors, ZINC000096928979 (Deleobuvir), and ZINC000003974230 ligands.

ADME Properties and Toxicity Profiles						
Properties		**Best Scored Inhibitors**			**Best Scored Ligands**	
	Ligand Name	Morusin	SC75741	Neferine	ZINC00009692897 9 (Deleobuvir)	ZINC00000397423 0
	Formula	C25H24O6	C29H23N7O2S2	C38H44N2O6	C34H33BrN6O3	C26H15Cl2NO5
Physico-chemical properties	**Molecular Weight (g/mol)**	420.45	565.67	624.77	653.57	492.31
	Molar Refractivity	121.83	160.76	188.02	174.90	127.60
	TPSA (topological polar surface area)	100 .13 Å²	173.24 Å²	72.86 Å²	114.93 Å²	80.75 Å²
Lipophilicity	**Log $P_{o/w}$ (iLOGP)**	3.77	3.18	5.21	3.93	3.13
	Log $P_{o/w}$ (XLOGP 3)	5.52	5.48	6.70	5.64	4.29
	Log $P_{o/w}$ (WLOG P)	5.16	5.32	5.35	6.63	3.98
	Log $P_{o/w}$ (MLOG P)	2.09	2.69	3.46	3.73	3.27
	Log $P_{o/w}$ (SILICO S-IT)	5.18	6.26	6.64	5.84	4.82
	Consensus Log *P*o/w	4.35	4.59	5.47	5.15	3.90
Solubility	**Log *S* (SILICOS-IT)**	-6.11	-9.74	-10.74	-9.40	-8.63
	SILICOS-IT Solubility (mg/ml)	3.22e-04	1.02e-07	1.12e-08	2.57e-07	1.14e-06
	SILICOS-IT Solubility (mol/l)	7.79e-07	1.80e-10	1.80e-11	3.94e-010	2.32e-09
	Solubility Class	Poorly Soluble	Poorly Soluble	Insoluble	Poorly soluble	Moderately soluble
Druglikeness	**Druglikeness**	-0.78	7.33	5.45	1.89	0.73
	Drug-score	0.29	0.15	0.23	0.21	0.12
Pharmacokinetics	**GI absorption**	High	Low	High	Low	High
	BBB permeant	No	No	No	No	No
	P-gp substrate	No	No	No	No	No
	CYP1A2 inhibitor	No	No	No	No	No
	CYP2C19 inhibitor	Yes	Yes	No	Yes	Yes
	CYP2C9 inhibitor	Yes	Yes	No	No	Yes
	CYP2D6 inhibitor	No	No	No	Yes	No
	CYP3A4 inhibitor	No	Yes	No	No	No
Toxicity	**Mutagenicity**	No	No	No	No	Yes
	Tumorigenicity	No	No	No	No	No
	Irritant Effects	No	No	No	No	No
	Reproductive Effects	No	Yes	No	No	Yes

Table 4. Structurally similar ligands with Valstar, Ergotamine, and Benzo[a]pyrene-7, 8-Diol observed during screening.

Structurally Similar Ligands			
Chemical Structure	**Ligand Name**	**Binding Affinity (kcal/mol)**	**Dataset Name**
 ZINC000011616852 (Valstar)	ZINC000095618662	-12.8	World-not-FDA Approved Drugs
	ZINC000004214612	-12.3	World-not-FDA Approved Drugs
	ZINC000011616852	-12.3	FDA Approved Drugs
	ZINC000028232755	-12.0	FDA Approved Drugs
	ZINC000150339052	-12.0	World-not-FDA Approved Drugs
	ZINC000068205977	-11.9	Drugs in Clinical Trials
	ZINC000150338912	-11.8	World-not-FDA Approved Drugs
	ZINC000150339055	-11.8	World-not-FDA Approved Drugs
	ZINC000256630457	-11.8	World-not-FDA Approved Drugs
	ZINC000163535243	-11.7	World-not-FDA Approved Drugs
	ZINC000245224599	-11.7	World-not-FDA Approved Drugs
	ZINC000049783788	-11.4	FDA Approved Drugs
	ZINC000049918329	-11.4ğ	World-not-FDA Approved Drugs
	ZINC000256630463	-11.4	World-not-FDA Approved Drugs
	ZINC000028232750	-11.3	FDA Approved Drugs
	ZINC000049918330	-11.3	World-not-FDA Approved Drugs
	ZINC000004215812	-12.3	World-not-FDA Approved Drugs
 ZINC000052955754 (Ergotamine)	ZINC000052955754	-12.2	FDA Approved Drugs
	ZINC000003978005	-12.1	FDA Approved Drugs
	ZINC000053683151	-11.7	FDA Approved Drugs
	ZINC000003995616	-11.4	World-not-FDA Approved Drugs
	ZINC000030728718	-12.1	Non-human Metabolites
	ZINC000030728728	-12.0	Non-human Metabolites
	ZINC000030728723	-11.6	Non-human Metabolites
 ZINC000002019693 (Benzo[a]pyrene-7,8-Diol)	ZINC000030728707	-11.5	Non-human Metabolites
	ZINC000030728712	-11.4	Non-human Metabolites
	ZINC000002019693	-11.2	Non-human Metabolites
	ZINC000002019694	-11.2	Non-human Metabolites
	ZINC000002019692	-11.1	Non-human Metabolites
	ZINC000002019691	-11.0	Non-human Metabolites
	ZINC000030728694	-10.7	Non-human Metabolites
	ZINC000030728703	-10.4	Non-human Metabolites

Conclusion

Due to the fact that activation of the RelA subunit of NF-κB might be induced under various stresses, and it's responsible for regulation of the proliferation, apoptosis, and inflammatory genes, a strong connection between the activation of RelA and many proliferative, inflammatory, and muscle tissue diseases have been reported in the literature. As such, repurposable drugs and design novel inhibitors against RelA have potential to treat such diseases. Therefore, a novel Drug Library including 12,111 ligands was created and screened for the RelA protein. In addition, 16 commercially available inhibitors and the S-Adenosylmethionin (SAM) ligand found in chemical structure of the protein were analyzed with the same strategy. Results show that ZINC000096928979 (Deleobuvir), ZINC000012503187 (Conivaptan), and ZINC000003974230 ligands might be repurposed to stress based diseases progressed by RelA activation since they have high binding affinity through interactions with common amino acids recognized by the inhibitors, sufficient ADME properties and toxicity properties. Furthermore, 16 structurally similar ligands with Valstar, 5 structurally similar ligands with Ergotamine, and 11 structurally similar ligands with Benzo[a]pyrene-7,8-Diol were discovered. These findings demonstrate that the structures of the ligands might be utilized as scaffolding in further structure based drug design studies. Therefore, the ligands with high potential to be

used in the treatment of RelA based diseases should be tested both in vitro and in vivo applications, and the stabilities of the ligands should be verified with further moleculer dynamics (MD) simulation studies.

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