Bioactive Agents Contained in Different Nasal Sprays May Defeat SARS-Cov-2: A Repurposing and In-Silico Approach

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Abstract

Recently, Coronavirus Disease 2019 (COVID-19), caused by fast-spreading and highly contagious severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has been declared as a pandemic disease of the 21st century by the World Health Organization (WHO). SARS-CoV-2 enters into the human respiratory system by binding of the viral surface spike glycoprotein (S-protein) to angiotensin-converting enzyme2 (ACE2) receptor that is found in the nasal passage and oral cavity of a human. Both spike protein and the ACE2 receptor have been identified as promising therapeutic targets to develop anti-SARS-CoV-2 drugs. Although in the last few months, various studies have identified some promising molecules against both the receptors including human ACE2 and SARS-CoV-2 spike protein, still there is no vaccine or therapeutic drugs as of today. The repurposing of FDA-approved drugs may provide a rapid and potential treatment to combat COVID-19 by using high throughput virtual screening approach. In the present study, we have used the repurposing approach for bioactive agents of the nasal spray against human ACE2 and SARS-CoV-2 spike protein to identify the anti-COVID-19 agents with the help of molecular docking study. To this, we screened the sixteen bioactive agents of the nasal spray by analyzing their binding free energy and binding mode through molecular docking study. As a result, bioactive agents such as ciclesonide, levocabastine, and triamcinolone acetonide were found as highly active ligands with potent binding affinities against both the targets human ACE2 and SARS-CoV-2 spike proteins. Thus, these bioactive agents may effectively assist to control the COVID-19 by inhibiting the human ACE2 receptor as well as spike protein of SARS-CoV-2.

Introduction

Since the first outbreak in Wuhan, China, the novel coronavirus disease 2019 (COVID-19) is still an unprecedented respiratory health problem, as of today 24th December 2020, causing the death of more than 30 million people globally. COVID-19 is caused by the severe acute respiratory coronavirus 2 (SARS-CoV-2), a single-stranded RNA-enveloped virus that enters into the human body mainly through the nose. SARS-CoV-2 also enters through the mouth but to a lesser extent [1]. The virus invades the nasal and oral passage through the binding of its surface spike (S) protein with the human angiotensin-converting enzyme 2 (ACE2) receptors and other targets [2]. After enters into the human, the virus then synthesizes RNA via its RNA-dependent RNA polymerase which leads to the formation of main protease and complete viral assembly. Once completion of assembly, viral particles release into the lower respiratory tract as well as in the external environment. In the respiratory system, they cause mild respiratory symptoms, but, in some cases, intense inflammatory host response may be triggered, leading to a life-threatening acute respiratory syndrome [3]. Thus, SARS-CoV-2 resides in the nasal and oral passages and then propagates around in the environment. Medical practitioners, especially front-line health workers are at higher risk of getting the infection through direct contact or aerosol transmission of viral particles. From all over the world, researchers and health practitioners are working incessantly to understand and to find out the cure for this deadly respiratory disease. By the date of writing this study, no specific drug or vaccine has been

found against COVID-19. Numerous treatments and suggestions including the use of surface disinfectants, social distancing, mask, and implementation of herbal/traditional medicines have been studied and published to prevent SARS-CoV-2 transmission [4, 5]. Therefore, effective treatments are in urgent need.

New drug discovery or therapeutic treatment against any disease is a difficult task because it takes a long time up to 14-15 years, and expenses more than a billion dollars along with a low success (2.01%) rate [6]. Thus, these challenges are stand up like a hard wall for the development of a new therapeutic agent to combat the COVID-19 globally. Keeping the time and cost in view, the repurposing approach of existing drugs may be a useful strategy to fight against SARS-CoV-2. In a recent outbreak, repurposing of available drugs is the only best option because of using this approach; it becomes easy for known therapeutic agents that are already FDA-approved drugs or the preclinical agents, which can directly enter in human clinical trials. Moreover, repurposing drugs approach can boost up the drug development process with the very low cost within a short period for the treatment of COVID-19.

Nasal sprays are liquid medicines that are delivered locally in the nasal cavity to help relieve acute or chronic congestion (stuffiness), rhinitis, common cold, sinusitis, hay fever, or other types of allergies [7]. Various types of nasal sprays are available in the market as antihistaminic solutions to reduce the inflammatory effects, or as steroid solutions to relieve the symptoms of common cold, sinusitis, hay fever, allergic rhinitis, and other allergies, or as salt-solution (also referred to as saline nasal spray) that can loosen up the mucous or as topical decongestant solutions to constrict the blood vessels at the time of inflammation process [8, 9]. Several studies showed that the bioactive agents contained in different nasal sprays have the ability to deactivate the virus within a few seconds. During the condition of the common cold, millions of viruses are present in the nasal passages that are transmitted from person to person. The use of nasal spray inhibits the transmission cycle of the virus and reduce the symptoms of common cold [10]. Hypertonic saline solution (NaCl) has an antiviral effect via reduction of viral load in the upper respiratory tract region [11]. Numerous repurposing anti-viral agents namely Remdesivir, Favipiravir, Nafamostat, Chloroquine, and Hydroxychloroquine, etc. have been studied for their in-vitro and in-vivo anti-SARS-CoV-2 activity, but no study is available on the repurposing of bioactive agents of the nasal spray against SARS-CoV-2. In a study, oxymetazoline and xylometazoline have been found to show reduce rhinovirus infections [12]. In addition, povidone-Iodine containing nasal spray to reduce the nasopharyngeal viral load in patients with COVID-19 is in under clinical trials. [13]. Thus keeping in mind, we, herein, hypothesized that the use of nasal spray will reduce the COVID-19 transmission in nasal passages through inhibition of SARS-CoV-2. In particular, sixteen bioactive agents of different nasal spray solutions that are already approved by various regulatory authorities including the U.S. Food and Drug Administration (FDA) as anti-inflammatory and anti-histaminic agents, have been screened by our research group against human ACE2 receptor and SARS-CoV-2 surface spike protein. As per the prediction, our study will provide useful data that can be utilized for preclinical and clinical studies to get mechanistic insights regarding the results of molecular docking and other computational experiments.

Material And Methods

Hardware and software information

All computational studies were performed on a computer cluster system provided by Gigabyte Technology co., LTD, model-B365M DS3H running Intel Core i5-9400F CPU @2.90GHz Processor, 16 GB RAM, 1TB hard disk, and NVIDIA Graphic Card. We have used the AutoDock Vina and PyMol software for the molecular docking study and the visualization of protein-ligand complexes, respectively.

Selection of ligands

Structure-based repurposing of clinically approved bioactive agents of different nasal spray solutions was selected for screening to find out the potent antiviral agent. The chemical structures, as shown in figure 1, of bioactive agents as ligands were retrieved from the PubChem site including fluticasone (PubChem ID: 5311101), fluticasone furoate (PubChem ID: 9854489), fluticasone propionate (PubChem ID: 444036), mometasone (PubChem ID: 441336), mometasone furoate (PubChem ID: 441336), oxymetazoline (PubChem ID: 4636), xylometazoline (PubChem ID: 5709), azelastine (PubChem ID: 2267), beclomethasone dipropionate (PubChem ID: 21700), budesonide (PubChem ID: 5281004), ciclesonide (PubChem ID: 6918155), flunisolide (PubChem ID: 82153), levocabastine (PubChem ID: 54385), olopatadine (PubChem ID: 5281071), phenylephrine (PubChem ID: 6041), triamcinolone acetonide (PubChem ID: 6436). The geometries of all ligands were optimized with the help of Discovery Studio 3.0 by using a clean geometry option and the CHARMm (MMFF94) force field was applied on the ligand molecules saved into .sdf into .pdb format. The ligand preparation was done by detecting root, set number of torsions, and aromaticity criteria using AutoDock Tool.

Selection of receptors

For the docking of ligands, the 3D-crystal structures of human angiotensin-converting enzyme (ACE2) (PDB ID: 1R4L) and spike protein of SARS-CoV-2 (PDB ID: 6LZG) were used and retrieved from the protein data bank (PDB) https://www.rcsb.org/ website. The binding sites were also retrieved and performed using Discovery Studio 3.0 by removing Hetatm and adding a hydrogen atom to the target protein receptors. Furthermore added polar hydrogen atoms and Kollman charges for protein optimization.

Molecular Docking

Molecular docking studies of selected ligands into target receptors (1R4L and 6LZG) were performed using AutoDock Vina. The molecular docking study was prepared by receptor grid-box was generated by putting the binding site at coordinates with dimensions of 50 Å × 50 Å × 50 Å with a suitable spacing around target residues for 1R4L protein. Similarly, a grid box for 6LZG was also set with dimensions of 50 Å × 50 Å × 50 Å centering on target residues and empirical scoring function by using a flexible method were used for docking. Finally, the docking results were analyzed by using the PyMol visualizer and LigPlot.

Table 1: Binding energy and molecular interactions of bioactive agents contained in different nasal spray solutions against human ACE2 (1R4L) and spike protein (6LZG) of SARS-CoV-19

Entry	Ligands	Receptors	Binding Energy	Interacting Amino acids
1	Fluticasone	1R4L	-9.1	Asp269, Arg273, Phe274, Thr276, His345, Pro346, Asp367, Thr371, His374, Glu375, His378, Glu402, His505, Tyr515
		6LZG	-7.6	Gln98, Gln102, Tyr196, Tyr202, Trp203, Gly205, Asp206, Glu208, Val209, Lys562
2	Fluticasone furoate	1R4L	-9.8	Glu145, Asp269, Arg273, Phe274, His345, pro346, Cys361, Lys363, Asp368, Leu370, Thr371, His374, Glu406, Thr445, Arg518
		6LZG	-8.3	Gln102, Lys187, Tyr196, Tyr199, Tyr 202, Trp203, Gly205, Asp206, Asp509, Tyr510, Ser511, Arg514
3	Fluticasone propionate	1R4L	-8.9	Leu95, Gln98, Ala99, Asp206, Glu208, Val209, Asn210, Gly211, Leu391, Asn394, Ala396, Pro565, Trp566
		6LZG	-7.3	Leu95, Gln98, Ala99, Gln102, Tyr202, Gly205, Asp206, Glu208, Lys562
4	Mometasone	1R4L	-9.2	Asn149, Asp269, Phe274, Thr276, Asp367, Leu370, Thr371, Ser409,
		6LZG	-7.6	Tyr127, Leu144, Glu145, Asn149, Asp269, Trp271, Arg273, Phe374, Phe504
5	Mometasone furoate	1R4L	-9.9	Asn149, Ala153, Asp269, Phe274, Thr276, Lys363, Leu370, Thr371, Glu406, Ser409, Thr445
		6LZG	-7.9	Phe40, Ala348, Asp350, His378, Phe390, Asn394, Glu398
6	Oxymetazoline	1R4L	-7.3	Phe274, Pro346, Asp367, Leu370, Thr371, Glu406, Thr445, Arg518
		6LZG	-7.0	Leu95, Gln98, Gln102, Tyr196, Gly205, Asp206, Val209, Ala396, Lys562, Glu564, Trp566
7	Xylometazoline	1R4L	-7.4	Phe274, Thr276, Asp367, Leu370, Thr371, Glu406, Ser409, Thr445
		6LZG	-6.8	Leu95, Gln98, Gln102, Tyr196, Gly205, Asp206, Val209, Lys562, Glu564, Trp566
8	Azelastine	1R4L	-9.7	Tyr127, Asn149, Ala153, Asp269, Trp271, Phe274, His345, Lys363, Asp367, Thr371
		6LZG	-7.8	Phe40, Trp69,Leu73, Asp350, Phe390, Leu391
9	Beclomethasone dipropionate	1R4L	-9.0	Tyr127, Leu144, Glu145, Asn149, Trp271, Arg273, Cys344, His345, Thr371, His374, Glu406
		6LZG	-7.7	Phe40, Ser43, Ser44, Ala348, Trp349, Asp350, Pro346, Asp367, Asp368, Leu370, Thr371, His374, Glu406, Arg518

10	Budasonide	1R4L	-9.5	Tyr127, Trp271, Arg273, Phe274, His345, Pro346, Asp367, Asp368,Leu370, Thr371, His374, Glu406, Arg518
		6LZG	-8.4	Gln102, Lys187, Tyr196, Tyr199, Tyr202,Trp203, Asp206, Asp509, Tyr510, Ser511, Arg514
11	Ciclesonide	1R4L	-10.3	Asn149, Ala153, Asp269, Arg273, Phe274, Pro346, Asp368, Thr371, His374, Glu375, His378, Glu402, Glu406, His505, Tyr515, Arg518
		6LZG	-9.3	Phe40, Ser43, Ser44, Trp69, Ala348, Asp350, His378, Asp382, Phe390, Arg393, Asn394, His401
12	Flunisolide	1R4L	-9.5	Leu95, Glu98, Ala99, Glu208,Val209, Asn210, Asn394, Lys562, Pro565
		6LZG	-8.5	Gln102, Tyr196, Tyr199, Tyr202, Trp203, Asp206, tyr510, Ser511, Arg514
13	Levocabastine	1R4L	-9.7	Phe40, Thr347, His345, Ala348, His378, Tyr385, Asn394, Glu402, Phe504, Tyr510, Arg514, Tyr515
		6LZG	-9.0	Phe40, Leu73, Ala99, Leu100,Gln102, Asp350, Phe390, Leu391, Arg393, Asn394
14	Olopatadine	1R4L	-9.2	Arg273,Phe274, Thr276, His345, Pro346, Met360, Arg367, Asp368, Thr371, His374, Thr445, His505, Tyr515, Arg518
		6LZG	-7.2	Leu95, Gln98, Ala99, Gln102, Tyr196, Gly205, Glu208, Val209, Asn210, Lys562, Glu564, Pro565
15	Phenylpherine	1R4L	-6.0	Thr347, Ala348, His378, Phe504, Tyr510, Arg514, Tyr515
		6LZG	-6.1	Leu95, Glu208, Val209, Asn210, Ala396, Lys562, Pro565, Trp566
16	Triamcinolone acetonide	1R4L	-10.0	Tyr127, Glu145, Trp271, Arg273, Phe274, His345, Pro346, Lys363, Thr371, His374, Glu406
		6LZG	-8.2	Asp30, Asn33, His34, Glu37, Pro389, Arg403, Glu406, Tyr505

Results And Discussion

The widespread contagious nature of COVID-19 has created both public health and economical challenges to the human being, which have led to attempts for drug design and discovery against the SARS-CoV-2 at various target sites. However, no therapeutic agent or vaccine is registered for the prevention and treatment of COVID-19 as of now. Numerous studies using computational tools have been performed and are believed to be of immense importance. Moreover, the repurposing of already FDA-approved drugs or drug molecules as the target therapeutic agents against this disease remains a point of interest due to their excellent efficacy for the treatment of other diseases.

Results for the binding energies of all the ligands as bioactive agents of various nasal sprays are summarized in table 1. In our high throughput molecular docking screening, we have selected a scale of best binding free energy with value >10.0 kcal/mol against ACE2 protein (1R4L) >9.0 kcal/mol against spike protein (6LZG) respectively. Particularly, we found three ligands namely, ciclesonide, levocabastine, and triamcinolone acetonide with more binding free energy as compared to the said scale. The three-dimensional (3D) docked poses showing maximum binding affinity of these ligands along with their corresponding two-dimensional (2D) interaction plots are depicted in Figures 2, 3, and 4. The 2D view displayed both hydrophilic (e.g. hydrogen bonds) and hydrophobic forces (e.g. Vander Waal and pi-pi interactions) within the binding pockets of target proteins. Figure 2b shows that ciclesonide binds firmly through seven hydrogen bonds with protein residues Arg273, His505, His345, and Tyr515 along with thirteen hydrophobic interactions with Asn149, Ala153, Asp269, Phe274, Pro346, Asp368, Thr371, His374, Glu375, His374, Glu402, Glu406, Arg518 residues of human ACE2 protein whereas figure 2d displays one hydrogen bond and eleven hydrophobic interactions of ciclesonide with Ser43 and Phe40, Ser44, Trp69, Ala348, Asp350, His378, Asp382, Phe390, Arg393, Asn394, His401 residues of SARS-CoV-2 spike protein, respectively. Moreover, levocabastine binds to the active sites of SARS-CoV-2 spike protein through different covalent interactions with Phe40, Leu73, Ala99, Leu100, Gln102, Asp350, Phe390, Leu391, Arg393, Asn394 residues as depicted in figure 3.

Furthermore, as shown in figure 4, we also found that triamcinolone acetonide stabilizes in the active sites of human ACE2 protein residues namely Tyr127, Glu145, Trp271, Arg273, Phe274, His345, Pro346, Lys363, Thr371, His374, Glu406 through four hydrogen bonds as well as eight different types of hydrophilic interactions. The estimated binding free energy of the remaining bioactive agents namely fluticasone, fluticasone furoate, fluticasone propionate, mometasone, mometasone furoate, oxymetazoline, Xylometazoline, Azelastine, beclomethasone dipropionate, budesonide, flunisolide, olopatadine, phenylephrine along with the interacting amino acids are listed in Table xx. If we compare all bioactive agents mentioned above in terms of binding free energy, it can be seen that ciclesonide has the most negative binding free energy with –10.3 kcal/mol against human ACE2 and -9.2 kcal/mol against SARS-CoV-2 spike proteins. However, levocabastine showed the binding free energy with -9.0 kcal/mol against SARS-CoV-2 spike protein, followed by -10.0 kcal/mol binding energy of triamcinolone acetonide against human ACE2 protein. Finally, remaining bioactive agents were also found to exhibit significant binding free energies ranges from -6.0 to -9.9 kcal/mol and -6.1 to -8.4 kcal/mol against human ACE2 (1R4L) and SARS-CoV-2 spike (6LZG) proteins respectively. Thus, the molecular interactions in docked pose noticeably demonstrate that the ligands ciclesonide, levocabastine, and triamcinolone acetonide bind via hydrogen bonds and hydrophobic interactions within the active sites of the human ACE2 and SARS-CoV-2 spike proteins that play an important role in the viral attachment virus with the human nasal surface.

Conclusion

In conclusion, all bioactive agents as mentioned above were predicted as potent inhibitors of both the targets including human ACE2 and SARS-CoV-2 spike protein. Based on the selected binding energy (>

-10.0 kcal/mol for ACE2 and > -9.2 kcal/mol for spike protein) scale, ciclesonide was found to have high binding affinities with both the target receptors whereas levocabastine was active against ACE2 receptor and triamcinolone acetonide significantly binds with the spike protein. As a result of the present study, it has been suggested that nasal spray may be used as potential therapeutic agents.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

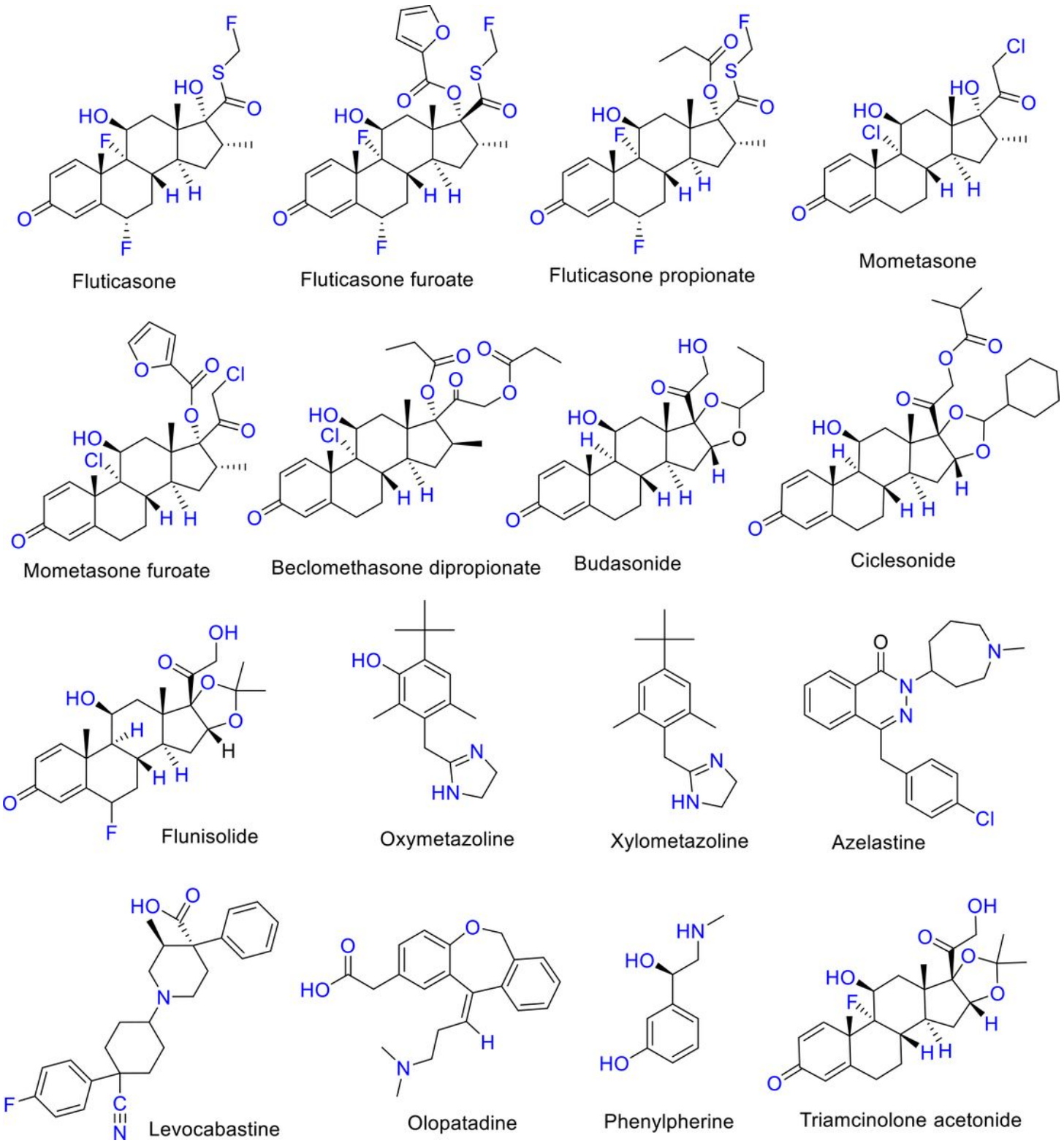

Figure 1

Chemical structures of bioactive agents from different nasal spray solution available in the market globally