Steroid Oximes

Subjects: Chemistry, Medicinal

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Steroids and their derivatives have been the subject of extensive research among investigators due to their wide range of pharmacological properties, in which steroidal oximes are included. Oximes are a chemical group with the general formula $R_1R_2C=N-OH$ and they exist as colorless crystals and are poorly soluble in water. Oximes can be easily obtained through the condensation of aldehydes or ketones with various amine derivatives, making them a very interesting chemical group in medicinal chemistry for the design of drugs as potential treatments for several diseases. A large number of steroid oximes exhibit important biological activities, such as anticancer, anti-inflammatory, antibacterial, antifungal and antiviral, among others, through different mechanisms of action. Several steroid oximes are used clinically as drugs and many others are in clinical trials.

Keywords: steroids ; oximes ; chemistry ; antitumor

1. Introduction

Steroids belong to a class of natural or synthetic organic compounds, whose basic molecular structure is typically composed of 17 carbon atoms, bonded in four “fused” rings: three six-member cyclohexane rings (rings A, B and C) and one five-member cyclopentane ring (the D ring). (**Figure 1**). They play a crucial role in the human body, being responsible for the regulation of several biological processes. This fact, together with their interesting biochemical properties, such as the ability to penetrate cell membranes and bind to the nuclear and membrane receptors, makes them extremely attractive in the design of new potential drugs for the treatment of several diseases [1][2]. In fact, since their discovery in 1935, steroids have been widely used in the treatment of several conditions in the most variable areas of medicine, for example, for the treatment of autoimmune and inflammatory diseases and for the treatment of cancer [3][4]. Given the privileged scaffold of steroids and their suitability for structural modifications, steroidal derivatives have been arousing interest among medicinal chemists in the hunt for novel drug candidates. Slight alterations in the basic ring structure of steroids can elicit an enormous change in biological activity, giving rise to steroidal derivatives with a wide range of therapeutic activities [5][6].

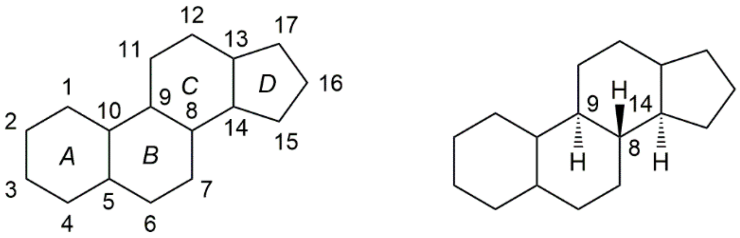

Figure 1. General structure of steroidal scaffold (left). Unless implied or stated to the contrary, the configuration of hydrogen atoms at the bridgehead positions 8, 9 and 14 are oriented as shown in the right formula (i.e., 8β, 9α, 14α).

Oximes are one of the most popular and extensively hailed nitrogen-containing biological compounds, presenting several biological and pharmacological applications [7]. They have achieved popularity due to their application as antidotes against nerve agents, which is attained by their capacity to reactivate acetylcholinesterase (AChE) [8]. Since that, these hydroxyimino derivatives have also been associated with several other biological activities such as antibacterial, antifungal, anti-inflammatory, antioxidant, and finally, anticancer as described [7][9].

Employing the hydrophobic steroid core with a hydroxyimino group constitutes an advantage since this chemical group can increase the molecules’ ability to interact with cell membranes, paving the way for enhanced biological activity [10]. For these reasons, in the last 20 years, a reasonable number of new steroidal oximes has been designed and synthesized and then evaluated for their biological activity.

2. Chemistry of Steroidal Oximes

Oximes ($R_1R_2C=N-OH$) (**Figure 2**) are a nitrogen-rich group of compounds, which are produced in nature in the plant and animal kingdom. In plants, oximes and their derivatives play a fundamental role in the metabolism of plant growth and development and in a variety of biosynthetic pathways [9][11]. In animals, oximes are most commonly known for their participation in the olfactory communication between animals [12]. Oximes exist as colorless crystals, are poorly soluble in water, and are easily accessible in laboratories and in industry, which makes them very appealing [13][14]. Additionally, they are extensively used not only as protectors of carbonyl groups but also as intermediates in the Beckmann rearrangement to synthesize several lactam derivatives [9][15][16]. Furthermore, oximes have the particularity of being easily transformed into different chemical groups such as amines, nitro, and other heterocyclic compounds [16][17].

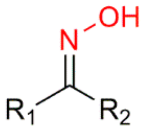

Figure 2. General structure of the hydroxyimino group of the oximes.

There are several ways to produce oxime derivatives and some reviews have been published regarding the chemistry of oximes [13][16][18]. The most classical method of oxime synthesis, which is the most used in the synthesis of steroidal oximes, involves the reaction of a carbonyl compound, a ketone or an aldehyde with hydroxylamine (NH_2OH) or a hydroxylammonium salt in the presence of a base (**Figure 3**). This type of reaction with aldehydes and non-symmetrical ketones can originate the two *E* or *Z* isomeric oxime forms, which can exist both as single compounds and or in mixture. Such chemical aspects can have a great impact on biological activity [7][12].

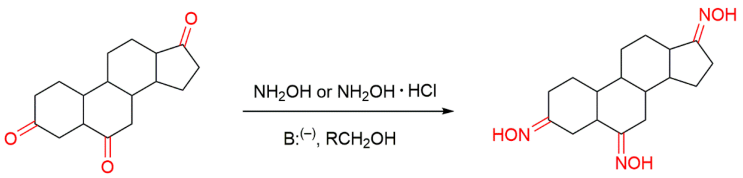

Figure 3. Classical synthesis of steroidal oximes which can take part in different positions in the steroidal scaffold.

Apart from the most commonly used synthetic strategy, there are other methods to prepare oximes involving non-carbonyl compounds, which consist of the reduction of nitroalkenes to create aldoximes and ketoximes. The reduction of α,β-unsaturated nitroalkenes gives rise to different oxime derivatives, depending if the nitro group is terminal or internal. More specifically, when the nitro group is terminal, aldoximes are produced, in mildly acidic conditions, in good yields. On the contrary, if the nitro group is internal, under basic conditions ketoximes are formed also in good yields [9][16]. Other variants of oxime synthesis include oxime ethers, esters, and amidoximes, all of which are of great biological and pharmacological importance. Oximes can act both as weak acids and weak bases. For this reason, the oxime anions can behave as ambident nucleophiles, which means that they can attack through two different sites, allowing them to be widely used for the synthesis of the above-mentioned class of compounds (ethers, nitrones, etc.) [13].

Another aspect of this chemical group is that it can behave both as hydrogen-bond donor (via OH group) and as hydrogen-bond acceptor (via nitrogen and oxygen atoms), which together with the high polarity of the oxime moiety can have a tremendous impact on the interaction with the receptor binding sites, enhancing biological activity, when compared to the carbonyl group [7][9].

3. Mechanisms of Action of Steroidal Oximes

Researchers came upon a wide variety of synthesized steroidal oximes, starting from androstane to estrane, pregnane, cholestane, diosgenin, and bile acids derivatives with very different mechanisms of action.

Most of the compounds summarized here are being evaluated as potential antitumor agents and exert their cytotoxicity against cancer cells mainly by inducing apoptosis; however, the pathways leading to it differ from compounds. For example, the androstane derivatives appear to induce apoptosis [19][20][21] by cell cycle arrest at different phases and increased ROS production [19]. Necroptosis might also be a mechanism involved in the compounds' cytotoxicity against cancer cells [19]. Oxime estrane derivatives induced cell death by apoptosis through cell cycle interference at G1 [22], S [23] and G2/M phases [24] and through activation of caspase-3 [22]. This class of compounds also interferes with microtubules by interfering with β-tubulin [24][25]. These are often related to cell cycle arrest and, consequently, a decrease in cell

division and cell death. Concerning oxime pregnane, diosgenin, and some cholestane derivatives, they cause cell cycle alterations, and cell death by the activation of the apoptotic intrinsic pathway mediated by caspase-3 activation [26][27] and release of cytochrome C [26][27][28]. Additionally, and since inflammation plays an important role in carcinogenesis, inhibition of pro-inflammatory genes such as TNF-α, COX, and IL6 is also another important mechanism displayed by these steroidal oximes [29]. Additionally, for some of the cholestane derivatives, their mechanism of action is not well elucidated since most of the studies were conducted mainly to infer about SAR, rather than go deeper into their mode of action. However, the breakthroughs reached in this field are of great importance and encourage the need to further analyze their mechanisms of action.

Aside from antitumor activity, steroidal oximes also display antimicrobial activity. However, little is known about the mode of action of these compounds. Regarding antiviral activity, it seems that steroidal oximes exert their effect by inhibition of the infection process of the virus after it enters the cells [30] and by a possible interaction with protein residues of heparan sulfate proteoglycan (HSPG) in host hepatocyte and bile acid receptor [31].

Concerning the compounds in clinical trials and already in clinical use, their mechanisms are very different. Istaroxime is an inhibitor of the Na^+/K^+ ATPase pump [32], whereas norgestimate and norelgestromin, which are already in the market for birth control, act as progesterone agonists, inhibiting ovulation [33][34].

4. Structure-Activity Relationships of Steroidal Oximes

The biological activity of a compound is closely related to its chemical structure. Steroidal oximes exert several types of biological activities, which can vary depending on the position of the oxime functionality on the steroid scaffold. Given this, some SAR can be elucidated especially for the antitumor and antimicrobial activity to help in the design and synthesis of novel molecules with pharmaceutical potential (**Figure 4**, **Figure 5** and **Figure 6**). However, the conclusions that can be drawn from **Figure 4**, **Figure 5** and **Figure 6** must be analyzed carefully, as they are just trends that were observed from the compounds, which were the most active compounds of each paper, and were evaluated with different methodologies, different times, and in different cancer cells.

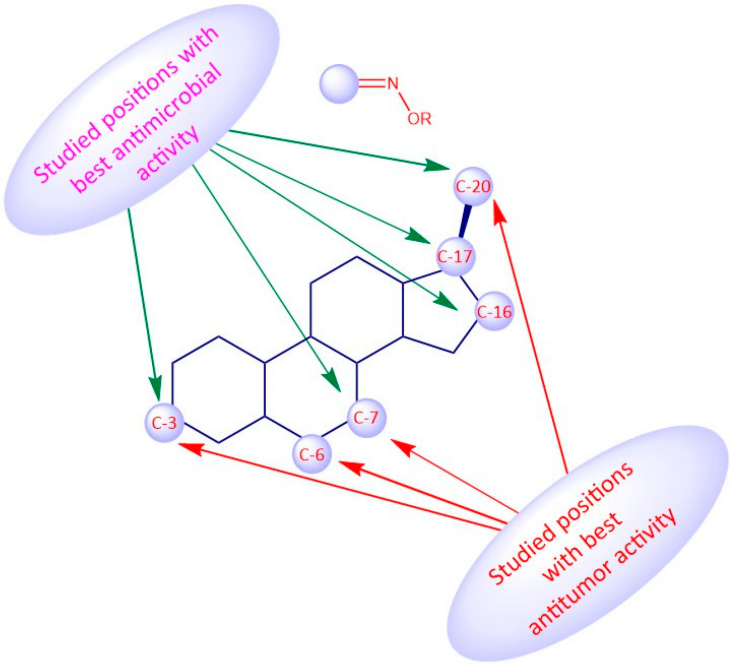

Figure 4. Overview of the most common SAR of steroidal oximes with antitumor and antimicrobial activity.

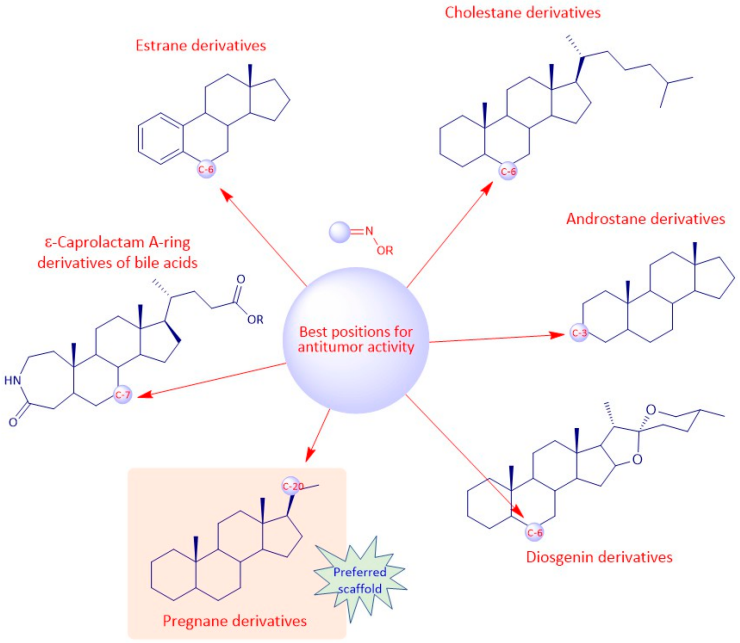

Figure 5. Most common SAR of steroidal oximes with antitumor activity.

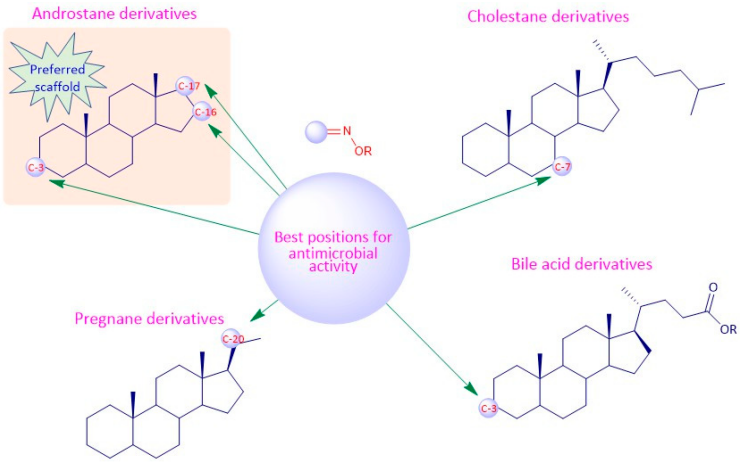

Figure 6. Most common SAR of steroidal oximes with antimicrobial activity.

Regarding the antitumor activity, when comparing the different IC_{50} values displayed by the androstane oxime derivatives, and despite the presence of other functional groups, it seems that position C-3 of the steroidal scaffold is preferable since the compounds with a hydroxyimino group in this position were, in general, the ones with the lower IC_{50} values (**1f**, **1g**, **1h**). Interestingly, the compounds that, in addition to having a hydroxyimino group at position C-3, also had another hydroxyimino group at position C-6 (**1b**, **1c** and **1u**) or at position C-17 (**1k**–**1o**) were slightly less active, suggesting that only a single hydroxyimino group at position C-3 is preferable and might be enough for the antitumor activity. Furthermore, in some types of cancer, namely in breast and prostate cancers, a hydroxyimino group at position C-2 was also important for the cytotoxicity displayed against cancer cells (compound **1r** and **1s**). Compound **1s** combines another hydroxyimino group at position C-4, which proved to be better than with just a single hydroxyimino group at position C-2. Positions C-17 and C-7 seem to be less favorable than the other positions mentioned above. Regarding the estrane series, position C-6 seems to be the most preferable concerning antitumor activity since compounds **2p** and **2q** were the ones with best IC_{50} values. In general, compounds with a hydroxyimino group at position C-17 were also very active. Concerning the pregnane series, all oximes (substituted or not) present high activity with very low IC_{50} values (IC_{50} values ranging from 0.31 to 7.17 μM. A hydroxyimino group at position C-6 appears to be the most advantageous position, regarding the cholestane series, since they present the lowers IC50s. With regard to the diosgenin series, the compounds with just a single hydroxyimino group were slightly more cytoxic against cancer cells than the ones containing two hydroxyimino groups (compounds **5b**, **5f** and **5g** present higher IC_{50} values than compounds **5a**, **5d** and **5e**). Finally, introduction of a hydroxyimino group in the B ring was clearly more beneficial than at C-ring, considering that compound **6a** presented a lower IC_{50} value than compound **6b**, for the case of bile acid derivatives.

Considering the antimicrobial activity, the androstane oxime derivatives with just a single hydroxyimino group were slightly more potent than the compounds with two hydroxyimino groups, suggesting that a single hydroxyimino group is better for antimicrobial activity rather than two hydroxyimino groups. Furthermore, a hydroxyimino group at position C-7 might be a

better option when designing and synthesizing oximes in the cholestane series, since the compounds with a hydroxyimino group at this position were slightly more toxic against pathogens than compounds with a hydroxyimino group at position C-6. Pregnane oxime derivatives were more active than the parent pregnane compounds.

To sum up, for the antitumor activity, SAR analysis points out that the pregnane scaffold might be the best option when designing novel molecules with antitumor activity. This steroidal oxime series presented the best IC_{50} values against the cancer cell lines studied when compared with the other above-mentioned series. As for the antimicrobial activity, and since the number of studies is considerably less, it turns out to be more difficult to establish robust SAR, but it seems that the androstane scaffold may be the most favorable option. In **Figure 4** researchers can see an overview of the most common SAR of steroidal oximes for the antitumor and antimicrobial biological activities.

References

1. Bhatti, H.N.; Khera, R.A. Biological transformations of steroidal compounds: A review. Steroids 2012, 77, 1267–1290.
2. Gupta, A.; Kumar, B.S.; Negi, A.S. Current status on development of steroids as anticancer agents. J. Steroid Biochem. Mol. Biol. 2013, 137, 242–270.
3. Ericson-Neilsen, W.; Kaye, A.D. Steroids: Pharmacology, complications, and practice delivery issues. Ochsner J. 2014, 14, 203–207.
4. Rasheed, A.; Qasim, M. A Review of natural steroids and their applications. Int. J. Pharm. Sci. Res. 2013, 4, 520–531.
5. Bansal, R.; Suryan, A. A Comprehensive Review on Steroidal Bioconjugates as Promising Leads in Drug Discovery. ACS Bio Med Chem Au 2022, 2, 340–369.
6. Bai, C.; Schmidt, A.; Freedman, L.P. Steroid Hormone Receptors and Drug Discovery: Therapeutic Opportunities and Assay Designs. ASSAY Drug Dev. Technol. 2003, 1, 843–852.
7. Schepetkin, I.; Plotnikov, M.; Khlebnikov, A.; Plotnikova, T.; Quinn, M. Oximes: Novel Therapeutics with Anticancer and Anti-Inflammatory Potential. Biomolecules 2021, 11, 777.
8. Grob, D.; Johns, R.J. Use of oximes in the treatment of intoxication by anticholinesterase compounds in normal subjects. Am. J. Med. 1958, 24, 497–511.
9. Dhuguru, J.; Zviagin, E.; Skouta, R. FDA-Approved Oximes and Their Significance in Medicinal Chemistry. Pharmaceuticals 2022, 15, 66.
10. Chen, S.-R.; Shen, F.-J.; Feng, G.-L.; Yuan, R.-X. Synthesis and Anticancer Activity of 4-azasteroidal-20-oxime Derivatives. J. Chem. Res. 2015, 39, 527–530.
11. Sørensen, M.; Neilson, E.H.; Møller, B.L. Oximes: Unrecognized Chameleons in General and Specialized Plant Metabolism. Mol. Plant 2017, 11, 95–117.
12. Surowiak, A.K.; Lochyński, S.; Strub, D.J. Unsubstituted oximes as potential therapeutic agents. Symmetry 2020, 12, 575.
13. Aakeröy, C.B.; Sinha, A.S.; Epa, K.N.; Chopade, P.D.; Smith, M.M.; Desper, J. Structural Chemistry of Oximes. Cryst. Growth Des. 2013, 13, 2687–2695.
14. Canario, C.; Silvestre, S.; Falcão, A.; Alves, G. Steroidal Oximes: Useful Compounds with Antitumor Activities. Curr. Med. Chem. 2018, 25, 660–686.
15. Donaruma, L.G.; Heldt, W.Z. The Beckmann rearrangement. Org. React. 2011, 15, 1–156.
16. Ãbele, E.; Lukevics, E. Recent advances in the chemistry of oximes. Org. Prep. Proced. Int. 2000, 32, 235–264.
17. Bolotin, D.S.; Bokach, N.A.; Demakova, M.Y.; Kukushkin, V.Y. Metal-Involving Synthesis and Reactions of Oximes. Chem. Rev. 2017, 117, 13039–13122.
18. Elliott, M.C. Imines and Their N-Substituted Derivatives: Hydrazones and Other N,N-Derivatives Including Diazo Compounds. Cheminform 2005, 36, 469–523.
19. Gomes, A.R.; Pires, A.S.; Abrantes, A.M.; Gonçalves, A.C.; Costa, S.C.; Varela, C.L.; Silva, E.T.; Botelho, M.F.; Roleira, F.M. Design, synthesis, and antitumor activity evaluation of steroidal oximes. Bioorg. Med. Chem. 2021, 46, 116360.
20. Ajduković, J.J.; Jakimov, D.S.; Rárová, L.; Strnad, M.; Dzichenka, Y.U.; Usanov, S.; Škorić, D.; Jovanović-Šanta, S.S.; Sakač, M.N. Novel alkylaminoethyl derivatives of androstane 3-oximes as anticancer candidates: Synthesis and evaluation of cytotoxic effects. RSC Adv. 2021, 11, 37449–37461.

21. Ajduković, J.J.; Penov Gaši, K.M.; Jakimov, D.S.; Klisurić, O.R.; Jovanović-Šanta, S.S.; Sakač, M.N.; Aleksić, L.D.; Djurendić, E.A. Synthesis, structural analysis and antitumor activity of novel 17α-picolyl and 17(E)-picolinylidene A-modified androstane derivatives. Bioorg. Med. Chem. 2015, 23, 1557–1568.
22. Berényi, Á.; Minorics, R.; Iványi, Z.; Ocsovszki, I.; Ducza, E.; Thole, H.; Messinger, J.; Wölfling, J.; Mótyán, G.; Mernyák, E.; et al. Synthesis and investigation of the anticancer effects of estrone-16-oxime ethers in vitro. Steroids 2013, 78, 69–78.
23. Mernyák, E.; Fiser, G.; Szabó, J.; Bodnár, B.; Schneider, G.; Kovács, I.; Ocsovszki, I.; Zupkó, I.; Wölfling, J. Synthesis and in vitro antiproliferative evaluation of d-secooxime derivatives of 13β- and 13α-estrone. Steroids 2014, 89, 47–55.
24. Canário, C.; Matias, M.; Brito, V.; Santos, A.; Falcão, A.; Silvestre, S.; Alves, G. New Estrone Oxime Derivatives: Synthesis, Cytotoxic Evaluation and Docking Studies. Molecules 2021, 26, 2687.
25. Cushman, M.; He, H.-M.; Katzenellenbogen, J.A.; Varma, R.K.; Hamel, E.; Lin, C.M.; Ram, S.; Sachdeva, Y.P. Synthesis of Analogs of 2-Methoxyestradiol with Enhanced Inhibitory Effects on Tubulin Polymerization and Cancer Cell Growth. J. Med. Chem. 1997, 40, 2323–2334.
26. Huang, Y.; Cui, J.; Zheng, Q.; Zeng, C.; Chen, Q.; Zhou, A. 6-Hydroximino-4-aza-A-homo-cholest-3-one and related analogue as a potent inducer of apoptosis in cancer cells. Steroids 2012, 77, 829–834.
27. Sánchez-Sánchez, L.; Hernández-Linares, M.G.; Escobar, M.L.; López-Muñoz, H.; Zenteno, E.; Fernández-Herrera, M.A.; Guerrero-Luna, G.; Carrasco-Carballo, A.; Sandoval-Ramírez, J. Antiproliferative, Cytotoxic, and Apoptotic Activity of Steroidal Oximes in Cervicouterine Cell Lines. Molecules 2016, 21, 1533.
28. Krstić, N.M.; Bjelaković, M.S.; Žižak, Ž.; Pavlović, M.D.; Juranić, Z.D.; Pavlović, V.D. Synthesis of some steroidal oximes, lactams, thiolactams and their antitumor activities. Steroids 2007, 72, 406–414.
29. Zeferino-Díaz, R.; Olivera-Castillo, L.; Dávalos, A.; Grant, G.; Kantún-Moreno, N.; Rodriguez-Canul, R.; Bernès, S.; Sandoval-Ramírez, J.; Fernández-Herrera, M.A. 22-Oxocholestane oximes as potential anti-inflammatory drug candidates. Eur. J. Med. Chem. 2019, 168, 78–86.
30. Pujol, C.A.; Sepúlveda, C.S.; Richmond, V.; Maier, M.S.; Damonte, E.B. Polyhydroxylated sulfated steroids derived from 5α-cholestanes as antiviral agents against herpes simplex virus. Arch. Virol. 2016, 161, 1993–1999.
31. Wei, Z.; Tan, J.; Cui, X.; Zhou, M.; Huang, Y.; Zang, N.; Chen, Z.; Wei, W. Design, Synthesis and Bioactive Evaluation of Oxime Derivatives of Dehydrocholic Acid as Anti-Hepatitis B Virus Agents. Molecules 2020, 25, 3359.
32. Aditya, S.; Rattan, A. Istaroxime: A rising star in acute heart failure. J. Pharmacol. Pharmacother. 2012, 3, 353–355.
33. Bringer, J. Norgestimate: A clinical overview of a new progestin. Am. J. Obstet. Gynecol. 1992, 166, 1969–1979.
34. Henzl, M.R. Norgestimate. From the laboratory to three clinical indications. J. Reprod Med. 2001, 46, 647–661.