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Nitroso-Derivatives of Dihydropyridine Calcium Channel Blockers (CCBs)

INTRODUCTION

Dihydropyridine calcium channel blockers (CCBs) are identified by the suffix "-dipine". These drugs include, amongst others, lacidipine, manidipine, clevidipine, amlodipine, nifedipine, felodipine, nitredipine, nilvadipine, lacidipine, isradipine, nimodipine, and nisoldipine.

Whilst the difunctional amine in the dihydropyridine moiety could theoretically be nitrosated to give the respective nitrosamines (Fig. 1) there are no literature reports (Scifinder) for such a transformation.

Manidipine and lercanidipine have an additional amine (a tertiary amine) that can theoretically be nitrosated, and this will also be discussed herein.

The purpose of this document is to explain why these nitrosated materials would not be considered cohort of concern nitrosamines.

In addition and unrelated to the mutagenic potential of this class of chemicals, the nitrosamines of dihydropyridine CCBs would not reasonably be predicted to form within a drug product formulation.

ASSESSMENT

Lack of ability to synthesize nitroso-dihydropyridine CCBs

The EMA has clarified that "*If, despite extensive efforts, it becomes apparent that the relevant nitrosamine impurity cannot be synthesized, then this could be an indication that the nitrosamine either does not exist or that there is no risk of it being formed. In such cases, it may not be necessary to conduct confirmatory testing. This should be justified thoroughly on a case by case basis according to appropriate scientific principles. The justification could include relevant literature, information on structural/stereo-electronic features and reactivity of the parent amine, stability of the nitrosamine and experimental data to illustrate the efforts made to synthesize and to analyse the impurity*."[1]

Industry members, including contract manufacturing organizations (CMO) which specialize in custom synthesis of reference standards, have attempted to synthesize several nitroso-dihydropyridine CCBs. One example is the attempt to synthesize the putative *N*-nitroso-nifedipide under neutral conditions (alkyl nitrite) and classical acidic conditions ($NaNO_2$/acid). The results from these experiments led to no formation of *N*-

nitroso-nifedipide, and when using the classical conditions, aromatization of the dihydropyridine ring occurred, as expected. No nitrosation was reported to occur on the aromatic ring that was formed. Earlier literature reports support these experimental observations and revealed that treatment of dihydropyridine derivatives with $NaNO_2$ using acidic conditions, results exclusively in aromatization of the dihydropyridine ring.[2,3,4]

Attempts were also made to synthesize the nitrosamine derivatives of felodipine, amlodipine, lacidipine, and lercanidipine. These experiments included the use of $NaNO_2$ and p-TSA, or $NaNO_2$ and 50% HCI, or *tert*-butyl nitrite, all at room temperature. Under any of the attempted conditions, the putative nitroso-dihydropyridine CCBs derivatives could not be synthesized which aligns with the lack of corresponding literature for this type of transformation (c.f. Scifinder). It was reported that under the above mentioned reaction conditions, the dihydropyridine ring tends to aromatize without undergoing nitrosation or nitration during the oxidation reaction,[2] e.g. amlodipine predominantly aromatizes to EP Impurity D and no trace of the nitroso impurity was formed.

Metabolism of Nitroso-Dihydropyridine CCBs

The importance of the α-position and its involvement in the bioactivation of nitrosamines has been discussed in various reviews.[5,6,7,8] The mutagenic mechanism of action of potent nitrosamines is by α-hydroxylation and subsequent elimination of a carbonyl compound that leads to the formation of an alkyldiazohydroxide, which, on loss of a hydroxide, gives an alkyldiazonium or decomposes further to a carbonium ion. Both diazonium salt, or derived carbonium ion, are potentially capable of alkylating DNA (Scheme 1). Compounds that lack α-carbon hydrogens cannot follow this metabolic pathway and do not form the diazonium or carbonium DNA-reactive alkylating species.

The nitroso-dihydropyridine CCBs do not have α-carbon hydrogens and thus they cannot metabolize to give the necessary alkylating metabolite. There are also no other plausible mechanisms that would lead to the formation of a diazonium ion from these nitrosamines. Therefore, this class of nitrosamines lacks the mechanism for potent toxicity of nitrosamines and can be considered non-cohort of concern impurities.

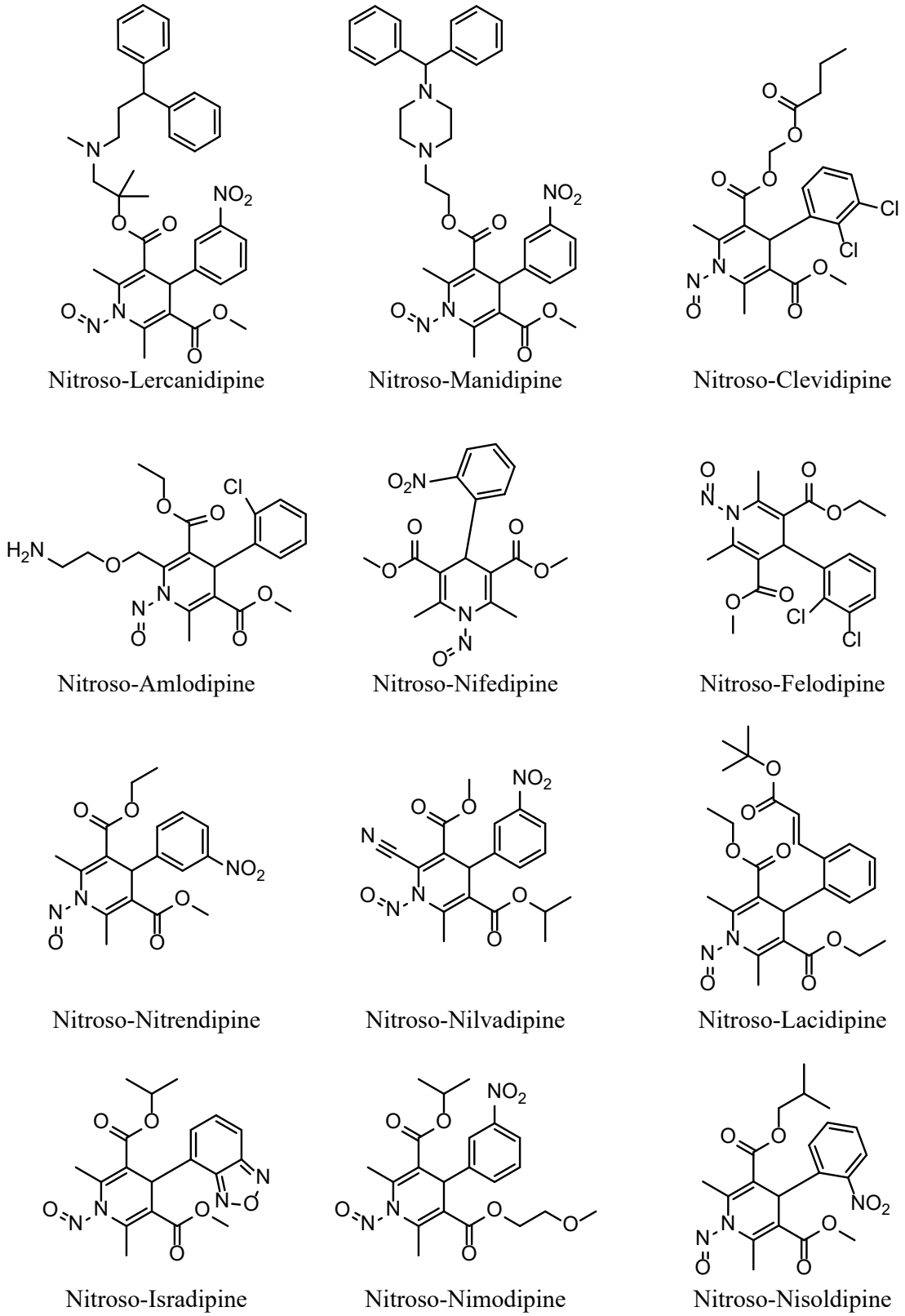

Figure 1. Structures of nitroso-dihydropyridine CCBs

Scheme 1. Metabolic activation of nitrosamines to generate a DNA-reactive alkylating agent.

Nitrosation of Tertiary Amines in CCBs

The tertiary amine in manidipine and lercanidipine can theoretically be nitrosated, however, tertiary amines have lower propensity to nitrosate in comparison to secondary amines. Furthermore, the preference of the dihydropyridines to undergo oxidative aromatization to the pyridine rather than form a stable nitrosamine is a reasonably rapid process and will most probably out compete a tertiary amine side chain for trace nitrite (sodium nitrite has been shown to be critical for the oxidation reaction; without it the oxidation reaction is very slow[2,3]). In most probability, the nitrosative oxidation of the dihydropyridine will protect the tertiary amine from dealkylative nitrosation by effectively scavenging nitrite. Therefore, there is no expectation that the tertiary amines in manidipine and lercanidipine will dealkylate and nitrosate. Currently there are no pharmacopeial monographs for manidipine and lercanidipine, so we are not aware of any potential presence of secondary amines as impurities of these two tertiary amine APIs.

SUMMARY AND CONCLUSION

Dihydropyridine calcium channel blockers (CCBs) have no hydrogens at the α-carbon adjacent to the amino group within the dihydropyridine and thus metabolic activation to form the mutagenic species cannot take place.

Furthermore, it was experimentally shown that nitrosation of the dihydropyridine in dihydropyridine CCBs does not occur, and under acidic/nitrosative conditions there is exclusive formation of aromatic derivatives. This oxidative aromatization also probably protects the CCBs that have an additional tertiary amine from N-nitrosation.

In conclusion, under the final product manufacturing conditions there is negligible risk for formation of the nitrosamine derivatives of dihydropyridine CCBs, and even if they were to form, they would not be considered cohort of concern mutagens based on the lack of ability for them to metabolize to DNA alkylating species associated with potent N-nitrosamines.

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