Semicarbazide-Sensitive Amine Oxidase: Biochemical and Physiological Properties

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ABSTRACT

The semicarbazide-sensitive amine oxidase (SSAO) is an enzyme widely distributed in many organs of mammals. The functional role of SSAO is not yet quite clear, but it is suggested that it plays roles in protection against exogenous amines, glucose transport, apoptosis, atherogenesis, cell adhesion, local generation of hydrogen peroxide as signal molecule, cross-linking of proteins and leucocyte trafficking. Plasma SSAO is reported to be elevated in diabetes mellitus, congestive heart failure, Alzheimer’s disease and some inflammatory diseases. SSAO-mediated deamination of substrates produces formaldehyde and methylglyoxal, which have been proposed to be cytotoxic to the various tissues and might be involved in the pathogenesis of some diseases such as atherosclerosis, aging, cancer and skin disorders. Although SSAO has been known for years, its physiological and pathological implications are just beginning to be recognized. This review summarizes the molecular, functional and pathological properties of SSAO.

Key Words: Semicarbazide-sensitive amine oxidase (SSAO), oxidative deamination, xenobiotics, substrate, inhibitor.

ÖZET

Semicarbazid-duyarlı amin oksidaz (SSAO), memeli organlarında yaygın olarak bulunan bir enzimdir. SSAO’nun fizyolojik görevi henüz kesin olarak bilmemekte, ancak enzimin dış kaynaklı aminlere karşı korunmada, glukoz taşınmasında, apoptoziste, aterojenezde, hücre tutunmasında, bir sinyal moleküle olarak hidrojen peroksitideki rol oynaması, proteinlerin çapraz bağlı olması ve leucosit trafikinde rol oynadığı öne sürülmektedir. Plazma SSAO düzeyinin diyabette, doğuştan kalp yetmezliğinde, Alzheimer hastalığına ve bazı inflamatuar hastalıklara bağlı olarak yükseldiği bilinmektedir. Bazı substratların SSAO-katalizli deaminasyonu sonucu oluşan formaldehit ve metilglioksal’ın sitotoksik etki gösterdiği ve ateroskleroz, yaşlanma, kanser ve deri bozukluklarının patojenezine katkıda bulunduğunu ileri sürülmüştür. SSAO uzun yıllardan beri bilinmekle birlikte, enzimin fizyolojik ve patolojik etkinlikleri henüz tanımmaya başlanmıştır. Bu derleme, SSAO’nun moleküller, işlevsel ve patolojik özellikleri özetlemektedir.

Anahtar Kelimeler: Semicarbazid-duyarlı amin oksidaz, oksidatif deaminasyon, ksenobiyotikler, substrat, inhibitör.
1. INTRODUCTION

The oxidative deamination of endogenous and exogenous amines in mammals is catalyzed by a number of oxidases (1,2). Semicarbazide-sensitive amine oxidases (EC 1.4.3.6: amine:oxygen oxidoreductase (deaminating), SSAOs) are a group of enzymes containing copper and quinone and sensitive to semicarbazide (3,4). SSAO activity is found in a great variety of species from prokaryotes to eukaryotes, including human. The enzyme is shown to be present in cell membranes as tissue-bound form or located in the vascular system and in adipocytes as soluble form (5). Their physiological functions are yet not clear, but it has been postulated that SSAO may be involved in detoxifying xenobiotics, regulating glucose uptake, and effecting cell adhesion, leukocyte trafficking and angiogenesis (6-11). Increased plasma SSAO activities were reported in patients with diabetes, alcoholics, Alzheimer’s disease, heart and vascular diseases (12-16). Although SSAO has been mostly regarded as being involved in the detoxification of amines, the products of the reaction are more toxic than the amine substrates themselves (17,18). Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), formaldehyde and methylglyoxal, simultaneously formed during deamination of the substrates, such as methylvamine and aminoaceton by SSAO, were reported to lead to increased oxidative stress, protein cross-linkage and cytotoxicity (16-20). Thus, SSAO may be responsible for vascular damage, atherosclerosis, diabetic complications, Alzheimer’s disease and aging via these mechanisms.

The aim of the present review is to briefly overview the biochemical properties and physiological functions of SSAO and to discuss its possible role in certain diseases.

2. MOLECULAR PROPERTIES OF SSAO

Amine oxidases are key enzymes which are widely distributed in nature and play important roles in the metabolism of biogenic amines (21). Monoamine oxidase (MAO), a FAD-dependent amine oxidase, which plays an essential role in the oxidative deamination of biogenic amines such as serotonin, dopamine, adrenaline and also catalyzes the oxidation of xenobiotic amines has been extensively characterized (22), whereas, little is known about the structure and function of SSAO, copper-containing amine oxidase (Table 1). These two enzymes are distinct from each other with respect to their substrate specificities and inhibitor sensitivities (17,23).

In mammals, SSAO is located in many organs and tissues, most prominently in vascular smooth muscle, adipocyte, cartilage, gut, lung, liver, retina, kidney, placenta, pancreas and plasma. It is absent from the nerves and glial cells of brain, but present in the microvessels of brain and thus may contribute to the blood-brain barrier (24). However, it has been suggested that it may be associated with the nerves of dental pulp (25). The enzyme exists in tissue-bound and soluble forms, but there are wide species and tissue differences in SSAO activities (24). Tissue-bound SSAO contains a short intracellular domain, a single transmembrane domain and a long extracellular domain which includes the catalytic site (26). Plasma SSAO is accepted to be originated from the cleavage of membrane-bound form. The sources of plasma SSAO is still unclear, but it is suggested that it may be derived from liver, retina, placenta and bone tissues (24,27,28).

The mammalian SSAO (180,000 Da) is a dimeric, glycosylated protein which contains 1 mol of copper per subunit. Cu (II) in SSAO was reported to be essential for the double hydroxylation of a tyrosine residue of SSAO with an autocatalytical reaction that yields the 6-hydroxydopa (TOPA) cofactor and also for providing a positive charge in the active site (26). It was shown that mammals contain two genes encoding SSAO, plus a pseudo-gene. One gene encodes the tissue-bound SSAO, the other encodes only one form exists in retina (29).

3. SUBSTRATE SPECIFICITY OF SSAO

The physiological substrates of SSAO include aminoaceton, methylamine, 2-phenylethylamine (PEA),

<table>
<thead>
<tr>
<th>Amine oxidase superfamily</th>
<th>Enzyme</th>
<th>Some substrates</th>
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<tbody>
<tr>
<td>FAD-dependent</td>
<td>Monoamine oxidase A</td>
<td>Dopamine, noradrenaline, serotonin</td>
</tr>
<tr>
<td></td>
<td>Monoamine oxidase B</td>
<td>Dopamine, phenylethylamine</td>
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<tr>
<td></td>
<td>Polyamine oxidase</td>
<td>Spermine, spermidine</td>
</tr>
<tr>
<td>Cu-dependent</td>
<td>Plasma SSAO</td>
<td>Aminoaceton, methylvamine, tyramine, benzylamine</td>
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<td></td>
<td>Tissue SSAO</td>
<td>Aminoaceton, methylvamine, tyramine, benzylamine</td>
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<tr>
<td></td>
<td>Diamine oxidase</td>
<td>Histamine, putrescine, Peptide-bound lysine residues</td>
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<td>Lysyl oxidase</td>
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tyramine and dopamine whereas benzylamine is a good non-physiological substrate for the mammalian SSAO (19,29). Although plasma SSAO usually has been termed as “benzylamine oxidase”, the physiological substrates of SSAO are accepted as aminoaceton, methylamine, 2-phenylethylamine, tyramine and dopamine (28-31). Most of the SSAO substrates are also oxidatively deaminated by MAO, but aminoaceton and methylamine are not MAO substrates (32). Serotonin (5-HT) is reported to be a good substrate for pig and human dental pulp SSAOs (25). SSAO also catalyses the oxidative deamination of a number of xenobiotics such as mescaline and anti-malarial drug, primaquine (33). Since the active site of SSAO is located in the extracellular domain (26,28), it seems that the enzyme is involved in the inactivation of potentially toxic amines in both tissues and blood. In contrast, monoamine oxidases are intracellular enzymes located in the mitochondrial outer membrane (34) and they are responsible for regulation and metabolism of major monoamine neurotransmitters such as serotonin, adrenaline, nor-adrenaline and dopamine (2) (Figure 1).

It is difficult to establish the substrate overlap between MAO and SSAO since tissue-bound SSAO activity possesses wide species differences in specificities and amount of enzyme present (17,28,30) (Figure 2). The levels of tissue-bound and plasma forms of SSAO vary widely between species and there are also differences in substrate specificities between SSAOs from different mammalian sources (12,24). For instance, mescaline is oxidised more efficiently than benzylamine by pig plasma SSAO while human SSAO does not show any activity towards this substrate (35). Stereospecificity also is important for the substrate affinity of the SSAO forms: oxidation of benzylamine by plasma SSAO from ox, horse, porcine, rabbit and sheep involves abstraction of the pro-S hydrogen whereas SSAO from human aorta and plasma shows no stereospecificity in this respect (36,37). It has been suggested that the structure of the copper-containing active site of different SSAOs detect the substrate specificity (38). Variations in glycosylation of SSAO, which differ between tissues and species, also effect the substrate specificity of SSAO (39).

4. SSAO-CATALYZED OXIDATIVE DEAMINATION

As shown in below, SSAO catalyze the oxidative deamination of substrates containing an amine moiety linked to an unsubstituted methylene group, which may be aliphatic or aromatic in nature. These substrates include dopamine, b-phenylethylamine, benzylamine, kynuramine, tryptamine, methylamine, allylamine and aminoaceton (3,17). An aldehyde metabolite, hydrogen peroxide and ammonia are produced by the deamination of RCH₂NH₂ substrate.

\[
\text{SSAO} \quad RCH₂NH₂ + O₂ + H₂O \longrightarrow RCHO + H₂O₂ + NH₃
\]

This reaction is a “ping-pong” reaction and can be divided into two separate half reactions as one reductive and one oxidative. In the first half reaction, the amine group
interacts with topa quinone co-factor (TPQ) in the active site and a Schiff base is produced. In the second half-reaction, the reduced TPQ is reoxidized by Cu(II) and O₂ under the H₂O₂ and NH₃ production (5):

\[
E-\text{CHO} + R\text{CH}_2\text{NH}_2 \rightarrow E\text{-CH}_2\text{NH}_2 + R\text{CHO} \\
E\text{-CH}_2\text{NH}_2 + O_2 + H_2O \rightarrow E\text{-CHO} + H_2O_2 + NH_3
\]

The membrane-bound SSAO is often characterized by its high affinity towards non-physiological amine, benzylamine, which is also a good substrate for MAO-B (40), indicating that SSAO and MAO overlap to some extent. However, SSAO is distinguished from MAOs by its insensitivity towards selective MAO inhibitors such as clorgyline, 1-deprenyl and pargyline (41). It has been recently shown that there is a sequence designated as -Asn-X-Asp-Tyr-Tyr- around TPQ, where X corresponds to SSAO, plays a vital role in SSAO-catalyzed deamination of substrates (39). TPQ co-factor was believed to be pyrroloquinoline quinone.

Methylamine and aminooacetone are readily deaminated by SSAO to yield methylglyoxal, formaldehyde, H₂O₂ and ammonia, both in vitro and in vivo (18,19,31,42).

SSAO

\[
\text{CH}_3\text{NH}_2 + O_2 + H_2O \rightarrow \text{HCHO} + \text{H}_2\text{O}_2 + \text{NH}_3 \\
\text{Methylamine} \quad \text{Formaldehyde}
\]

\[
\text{CH}_2\text{COCH}_2\text{NH}_2 + O_2 + H_2O \rightarrow \text{CH}_2\text{COCHO} + \text{H}_2\text{O}_2 + \text{NH}_3 \\
\text{Aminoacetone} \quad \text{Methylglyoxal}
\]

\[
\text{CH}_2 = \text{CHCH}_2\text{NH}_2 + O_2 + H_2O \rightarrow \text{CH}_2 = \text{CHCHO} + \text{H}_2\text{O}_2 + \text{NH}_3 \\
\text{Allylamine} \quad \text{Acrolein}
\]

Methylamine was found in blood, urine and tissues of humans (20,43) and can be derived from deamination of adrenaline, creatine and creatinine (44). Aminooacetone is endogenously derived from glycine or threonine (2,20). The aldehyde products of the SSAO reaction have attention in terms of their potential toxicity. These aldehydes may be oxidised to the corresponding carboxylic acid by aldehyde dehydrogenase or aldehyde oxidase or reduced to the corresponding alcohols by aldehyde reductases or alcohol dehydrogenase. However, formaldehyde produced by the oxidative deamination of methylamine is potentially toxic (43, 44). Since the formaldehyde produced would have to be transported into cells, such as erythrocytes for metabolism, this causes formaldehyde-induced toxicity in blood vessels (44).

Metabolism of xenobiotic allylamine by SSAO produces acrolein, which leads to vascular toxicity. It has been demonstrated that SSAO inhibition can prevent the SSAO-mediated vascular damage (45). It appears that this toxicity may result from the synergistic action of acrolein and H₂O₂, since the presence of catalase reduced the extent of the damage caused by allylamine oxidation (46).

Methylglyoxal cytotoxicity is resulted from its ability to cross-link of proteins and increased cross-linkage has been recognized to be involved in the aging process, which seems to be related to chronic vascular diseases (47).

H₂O₂ is a major reactive oxygen species, which is also generated in SSAO-catalyzed deaminations. H₂O₂ can be converted to toxic hydroxyl radical via the Fenton reaction and has been implicated in several diseases (48). Free radicals can be generated from formaldehyde in the presence of H₂O₂ under alkaline conditions, but it has been shown that in the presence of free amino group with formaldehyde and H₂O₂, however, excited formaldehyde and singlet oxygen are generated even under physiological conditions (49). It seems possible that SSAO-mediated oxidative stress may cause the oxidation of LDL and glycoxidation of proteins.

5. SSAO AND PATHOLOGICAL CONDITIONS

SSAO activity is found to be altered in a number of disease states, as summarized in Table 2. Plasma SSAO activity is increased in cardiac disease and in congestive heart failure (4,5,8,12,16,20,45). Atherogenesis is a complex process in which lesions formed at the blood vessels progress via fatty streaks, followed by formation of fibrous plaques and trombus, resulted in deposition of fibrin and plateletes. Atherogenesis involves endothelial disfunciton, smooth muscle proliferation and subsequ-

<table>
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<tr>
<th>Disease</th>
<th>Increased</th>
<th>Decreased</th>
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<tr>
<td>Cardiac disease (plasma)</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Congestive heart disease (plasma)</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Diabetes type II (human plasma)</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Diabetes (rat kidney)</td>
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<td>-</td>
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<tr>
<td>Diabetic retinopathy (plasma)</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Diabetic atherosclerosis (plasma)</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Diabetic nephropathy (plasma)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hypertension (plasma)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Alzheimer’s disease (cerebral blood vessels)</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Burns(plasma)</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Cancer (solid tumour) (tumour tissue)</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Cancer (breast) (plasma)</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Inflammatory liver disease (plasma)</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Kidney transplant rejection (plasma)</td>
<td>+</td>
<td>-</td>
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<td>Pre-eclampsia (plasma)</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Stroke (plasma)</td>
<td>-</td>
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</tr>
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</table>

Table 2. Altered SSAO activity in some diseases

Turk J Biochem, 2004; 29(3); 247-254.
ently, disruption. Hypotheses regarding the mechanism of atherogenesis include oxidative stress, hypercholes-
terolemia, LDL, LDL receptors, Apo-E, advanced glycation, cytokines, hormones, abnormal lipid meta-
bolism, etc. It has been suggested that SSAO-mediated deamination is involved in atherogenesis and vascular
disorders and selective SSAO inhibitors can prevent such toxicity (44,46). Formaldehyde and H₂O₂ derived
from SSAO-catalyzed methylamine deamination, or increased availability of substrates have been proposed
to cause chronic stress; damage endothelial cells; indu-
ce protein cross-linkage of structural proteins, such as
collagen; increase rigidity of blood vessels and lead to
vascular dysfunction (17,46). Allylamine is reported to
cause extensive and progressive vascular and myocardi-
al lesions similar to that seen in atherosclerosis and this
vascular toxicity of allylamine can be prevented by the
SSAO inhibitor semicarbazide (13,46).

SSAO expression was shown to be increased in cerebral
blood vessels of subjects with Alzheimer’s disease (13,
30,50). Aldehydes produced by SSAO-mediated deami-
ation of methylamine and aminoacetone were suggested
to cause intra- and intermolecular protein cross-linkages
and b-amyloid formation, deposition and subsequently
plaque formation in the compartments adjacent to the
cerebrovessels (51). Since SSAO-mediated generation of formaldehyde can also lead to cytotoxicity, which
induces inflammation and release of more SSAO, it has
been postulated that increased SSAO-mediated reaction
may be chronically involved in the pathogenesis of vas-
cular dementia (51).

Already in the 1960s it was demonstrated that plasma
SSAO activity was elevated in patients with diabetes
mellitus (4,5,8,10,14) and recently it was shown that
this increase in activity is correlated to the degree of
vascular damage, nephropathy, and retinopathy (18,52,
53). Increased SSAO activity has been observed in sheep
and rat plasma and rat kidney in experimental diabetic
models (54). These observations have been further con-
firmed in both Type I and II diabetics (7,8,10,14,52).
Formaldehyde and H₂O₂, derived from SSAO-mediated
methylamine deamination, were found to be responsible
for the diabetic complications (5,8,14,18,53). SSAO is
known to be selectively located in tissues which are vul-
nerable to diabetic complications and it can be released
into the blood stream from damaged SSAO-rich tissues.
Interestingly, the sequence of another protein called
VAP-1 has been found to be identical to SSAO which
has been shown to be capable of deaminating amines.
VAP-1 induces cell adhesion and regulates lymphocyte
trafficking and it was reported that it is involved in gra-
nuocyte extravasation and inflammation (9,55). Thus,
it seems possible that this protein is the same protein as
SSAO and increased expression of it as a response to in-
flammation leads to enhanced levels of toxic aldehydes
in blood, increased oxidative stress and cause vascular
injury and inflammation (55).

SSAO has been found to be involved in the regulation of
GLUT-4 in isolated rat adipose cells (7). Benzylamine,
an SSAO substrate, caused a marked stimulation of
glucose uptake in adipocytes and this induction was
blocked by catalase and SSAO inhibitors suggesting that
H₂O₂ production resulted from SSAO-mediated
deamination plays a crucial regulatory role in this pro-
cess (7). SSAO has been claimed to be an important role
in glucose uptake in adipocytes since SSAO-mediated
deamination mimics insulin-like actions such as signal
transduction, lipid metabolism and differentiation of
adipocytes (56).

6. ALTERNATIVE FUNCTIONS OF
SSAO

Although products of SSAO-catalyzed deaminations are
potentially toxic, they may have important roles in some
certain physiological conditions. Hydrogen peroxide
is known to mimic the effects of insulin and induces a
recruitment of intracellular GLUT-4 receptors to cell
surface, stimulates glucose uptake. SSAO substrates
have been shown to stimulate glucose transport and
SSAO inhibitors have been shown to stimulate glucose transport and
SSAO inhibitors abolish completely this effect (7). Since
activation of glucose transport was reversed by catalase,
it was suggested that H₂O₂ plays an important role in this
process (57).

SSAO activity appears to play a significant role in the
development of some cell types. Methylamine and other
SSAO substrates were shown to induce maturation of
adipocytes in a dose-dependent manner and since this
effect was prevented by SSAO inhibition and by tre-
ment with antioxidants, it was suggested that H₂O₂
formation plays a key role (56,58).

SSAO activity also plays an important role in extra-
cellular matrix deposition and maintenance in vascular
smooth muscle and inhibition of SSAO is resulted in
aberrations in collagen and elastin deposition by heart
smooth muscle cells (59).

VAP-1, which possess SSAO activity, is reported to sup-
port the adhesion of lymphocytes to endothelial cells and
mediates lymphocyte re-circulation and to be involved in
inflammatory conditions (55). VAP-1 has been shown to
support sialic-acid dependent adhesion under shear
stress and to mediate tethering to the tumour endothe-
lum in human heptacellular carcinoma of T-cells (60).
In mature adipocytes, SSAO is located in caveolae with
CD36 and the scavenger lipoprotein receptor as major
proteins, and may be involved in lipid transport (61).

7. SSAO INHIBITORS

Today, there are no selective and potent inhibitors of
human SSAO. Semicarbazide and cyanide are both
SSAO inhibitors that also inhibit some other enzymes. Some inhibitors of MAO, such as MDL 72145 ((E)-2-(3',4'-dimethoxyphenyl)-3-fluoroallylamine), originally developed as antidepressants, have been reported also to inhibit SSAO irreversibly (62). However, it is also a potent inhibitor of MAO-B and also affects MAO-A activity. Substituted b-chloroallylamines are weak inhibitors of MAO and MDL 72274 [(E)-b-phenyl-3-chloroallylamine] shows high potency and selectivity for SSAO in vitro, compared with its activity against MAO (63) (Figure 3). An inhibitor with high selectivity for pig plasma SSAO, named as B24, was synthesized as SSAO substrate, but appeared to be a highly potent SSAO inhibitor (35).

It has been shown that the primary aromatic monoamines with a single methyl substituent on a-carbon atom adjacent to amino group, are SSAO inhibitors with inhibitory properties of MAO, such as mexiletine and amphetamine (5). Amiflamine [FLA 336(+)], its enantiomer [FLA 336(-)] and its metabolites [FLA 788 (+), FLA 668 (+)] inhibit MAO-A and SSAO (64) (Figure 4). D,L-a-methylbenzylamine and its enantiomers D- and L-form of a-methylbenzylamine, are found to be SSAO and MAO-A inhibitors (65). 2-Bromoethylamine was shown to be a potent and selective SSAO inhibitor (66).

Hydrazine derivatives are also SSAO inhibitors (Figure 3). Highly selective SSAO inhibitor semicarbazide has already been introduced and detected as a useful compound for distinguishing SSAO from MAO in tissues (67). Some irreversible and non-selective MAO inhibitors, such as phenelzine, phenylhydrazine, hydralazine, aminoguanidine, iproniazide, isoniazide, nialamide, benzerazide and carbidopa, are thought to be possible SSAO inhibitors because of their abilities to bind to FAD in MAO which is outside of the substrate binding site (Figure 3). Hydralazine is a peripheral vasodilator used as anti-hypertensive and irreversible and partially time-dependent inhibitor of SSAO whereas phenylhydrazine is the potent irreversible SSAO inhibitor (68); aminoguanizine is used to prevent diabetic nephropathy (69).

Procarbazide and its metabolite monomethylhydrazine also appears to be highly selective for SSAO (70).

Fig. 3. Some hydrazine and haloamine derivatives presented as SSAO inhibitors.

Fig. 4. Some a-methylsubstituted amines designed as SSAO inhibitors.
8. CONCLUSION
SSAO was discovered over three decades ago during investigation of MAO. Little is known about its molecular structure and exact physiological functions in mammals, but it can be assumed that it may have several and competitive functions. A considerable amount of research evidence suggest that SSAO plays a role in vascular diseases, diabetes and Alzheimer’s disease. Increased knowledge regarding the structure of SSAO can facilitate the development of inhibitors with high potency and selectivity.

9. REFERENCES