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Orientation of oxazolidinones in the active site of monoamine oxidase

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Abstract

Oxazolidinone inhibitors of monoamine oxidase (MAO) and oxazolidinone antibacterials are two distinct classes of drug, often with linear structures and overlapping activities for some derivatives. By synthesizing novel dimerised derivatives with identical substitution of the two C-5 side chains, we have obtained experimental evidence for the orientation of oxazolidinones in the active site of MAO A. Two types of spectral changes, either increasing the absorbance at 510 nm or decreasing it at 495 nm depending on the group nearest to the flavin cofactor, were seen on ligand binding to MAO A. Side chain derivatives with amine substituents are very poor substrates so that it was possible to examine the spectral change due to binding of a substrate before reduction of the flavin occurred. Binding of these amino derivative substrates to MAO A induced a spectral change characterized by a strong decrease in absorbance at 495 nm. These substrates reduced the enzyme fully without any trace of a semiquinone intermediate. Only oxazolidinone inhibitors with a bromo-imidazole substituent increased the yield of semiquinone intermediate obtained during chemical reduction. In accord with the experimental data, results of docking experiments showed that binding of the oxazolidinone ring in the aromatic cage close to the flavin was favored and that the nitrogen of the derivatives that were substrates was within van der Waals distance of N-5 of the flavin.

Keywords: Oxazolidinone; Monoamine oxidase; Difference spectra; Flexible docking; Kinetics

1. Introduction

Monoamine oxidase A (MAO A; EC 1.4.3.4) is the key enzyme in the nervous system for catabolism of the catecholamine neurotransmitters and also in the intestine for scavenging ingested biogenic amines to prevent them reaching the circulation. MAO A catalyses the oxidation of a wide range of amines to the imine with concomitant reduction of the cysteinyl-FAD cofactor that is then reoxidised by oxygen producing H_2O_2.

The absorbance spectrum of the FAD in MAO A alters when ligands are bound [1–3]. The alteration is sensitive to minor changes in the ligand structure, so provides a tool for examining the interaction between inhibitors and the active site of MAO A. In this paper, we use this tool to determine differences in the interactions of oxazolidinone derivatives containing three different side chain substituents in the area expected to bind close to the flavin. The structure of MAO A is now available [4], so modelling of inhibitors into the active site was done to reveal the interactions responsible for the change in spectral and redox properties.

The proximity of the ligands to the flavin also influences the redox properties of the FAD in MAO A. In the absence of ligands, chemical reduction of MAO generates a spectrum characterized by a peak at 412 nm, typical of the anionic semiquinone form of the flavin (or possibly of a tyrosyl radical [5]), as an intermediate before full reduction. Inhibitors, such as D-amphetamine, increase the amount of intermediate seen and prevent full reduction [2,6,7]. In the presence of substrates, no intermediate is ever seen and reduction proceeds directly to the fully reduced form. Thermodynamic and kinetic studies have shown that substrates increase the redox potential of the flavin [8] and that substrate bound to the reduced enzyme accelerates the rate of reoxidation [9]. In contrast, inhibitors do not change the redox potential and they inhibit reoxidation. Since the substrate β-phenylethylamine differs from the inhibitor, D-amphetamine, by only a methyl

Abbreviation: MAO A, monoamine oxidase A

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group α to the amino group, this indicates that interactions close to the N-5 of the FAD may be responsible for the differences. Here, we look at the influence of acetamide, hydroxyl, and amine substituents on the yield of the semiquinone in MAO A.

Oxazolidinone derivatives are well-known inhibitors of both MAO A and MAO B [10–15]. They also include a new class of antibacterial agents that act by preventing protein synthesis by binding to the bacterial ribosome prior to initiation of synthesis [16]. The structure–activity relationships of the two activities are similar but differentiation can be made [14], so that this new class of antibacterials can be developed devoid of risk from the pressor response to tyramine or amines in the diet that can occur with irreversible MAO A inhibition [17,18]. Most oxazolidinones inhibit MAO A reversibly and competitively, although time-dependent inhibition of MAO B and irreversible inhibition have also been observed for some derivatives [19]. Oxazolidinones are linear heterocyclic molecules which, in principle, could bind in either orientation in the active site of MAO A. The dimerised derivatives **10**–**12** used in this investigation, shown in Fig. 1, were available from a separate study designed to assess the possibility of accessing adjacent antibacterial ribosomal binding sites, so their binding characteristics were compared with monomeric compounds known to interact with the active site of MAO A. In the event, they showed modest activity against the Gram-positive bacterial species *Staphlococcus aureus* and *Streptococcus pneumoniae* only with minimum inhibitory concentrations in the range of 4–8 mg/L.

Structure–function studies on MAO demonstrated that good binding is obtained with ligands that are generally lipophilic with an aromatic ring and a nitrogen (or oxygen or sulfur) close to the ring. Crystal structures of MAO A [4] and MAO B [20] have now provided a clear picture of the active site as a long, narrow hydrophobic cavity penetrating deep into the molecule to the flavin where the substrate is aligned by two tyrosines (Y407 and Y444 in MAO A) so that the amine is close to the N-5 of the flavin for catalysis. In this study, we provide experimental evidence for the orientation of oxazolidinones in this active site of MAO A. In addition, to seek an explanation for the ligand effects on

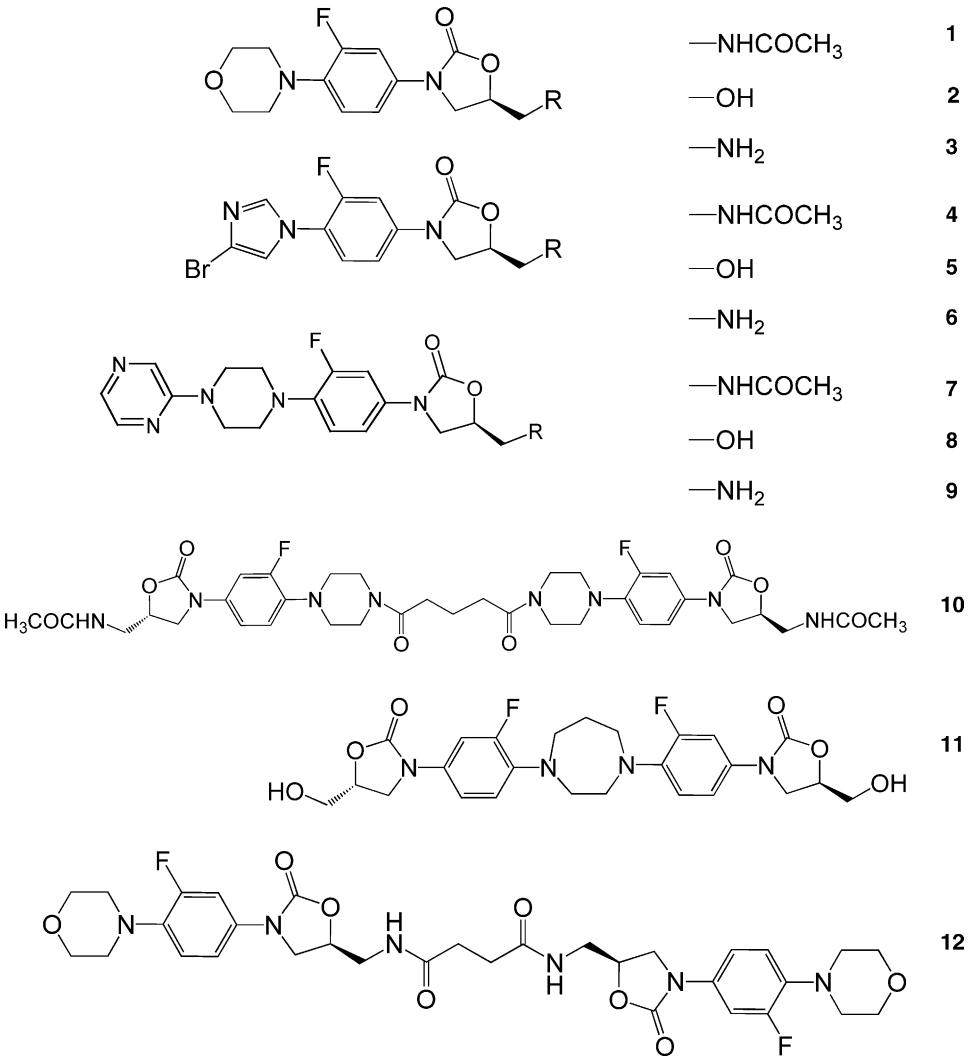

Fig. 1. Structures of oxazolidinone derivatives.

the flavin cofactor, seen as spectral changes on ligand binding and changes in the redox properties, we have docked the oxazolidinone derivatives in a model of the MAO A binding site based on its known structure.

2. Materials and methods

2.1. Materials

Monoamine oxidase A (human liver form) was purified after expression in *S. cerevisiae* [21]. The enzyme was stored at −20 °C in a solution of 50 mM potassium phosphate, pH 7.2, 0.8% *n*-octyl-β-D-glucopyranoside, 1.5 mM dithiothreitol, and 0.5 mM D-amphetamine, to which was then added an equal volume of glycerol. Before use, dithiothreitol, D-amphetamine, and glycerol were removed by gel filtration in a spin column of G-50 Sephadex equilibrated with 50 mM potassium phosphate, pH 7.2, containing 0.05% Brij.

The 4-(*N*-morpholino)-aryl, 4-(4′-bromo-*N*-imidazoyl)-aryl, and 4-(*N*′-2-(pyrazino)-*N*-piperazinyl)-aryl oxazolidinone derivatives were synthesized by known methods [22,23]. The novel dimer compound **10** (Chart 1) was synthesized by linking two equivalents of the known *N*-{[(5*S*)-3-(3-fluoro-4-piperazin-1-ylphenyl)-2-oxo-1,3-oxazolidin-5-yl]methyl}acetamide [22] with glutaryl dichloride to afford *N*,*N*′-((1,5-dioxopentane-1,5-diyl)-bis{piperazine-4,1-diyl(3-fluoro-4,1-phenylene)[(5*S*)-2-oxo-1,3-oxazolidine-3,5-diyl]methylene})diacetamide, compound **10**. Compound **11** was prepared (Chart 2) by a multi-step sequence beginning with the addition of homopiperazine to two equivalents of 3,4-difluoronitrobenzene to afford the dinitro compound. Reduction of the dinitro compound to the dianiline was accomplished using Pd/C in the presence of ammonium formate. The dianiline compound was then converted to its dibenzyloxycarbamate which was then treated with LiHMDS and *R*-(−)-glycidyl butyrate to afford (5*R*,5′*R*)-3,3′-[1,4-diazepane-1,4-diyl-bis(3-fluoro-4,1-phenylene)]bis[5-(hydroxymethyl)-1,3-oxazolidin-2-one], compound **11**. Compound **12** was prepared in an analogous manner to **10** but with amine **3** as the reactant and succinyl rather than glutaryl dichloride as acylating agent. All other chemicals were purchased from Sigma Chemical Company. All final compounds were isolated by standard procedures, purified by reverse phase chromatography and characterized by high resolution mass spectrum and 300 MHz NMR, which showed purities >98%.

2.2. MAO A assay and spectral titrations

Initial rates of oxidation were measured at 30 °C in 50 mM potassium phosphate, pH 7.2, containing 0.05%

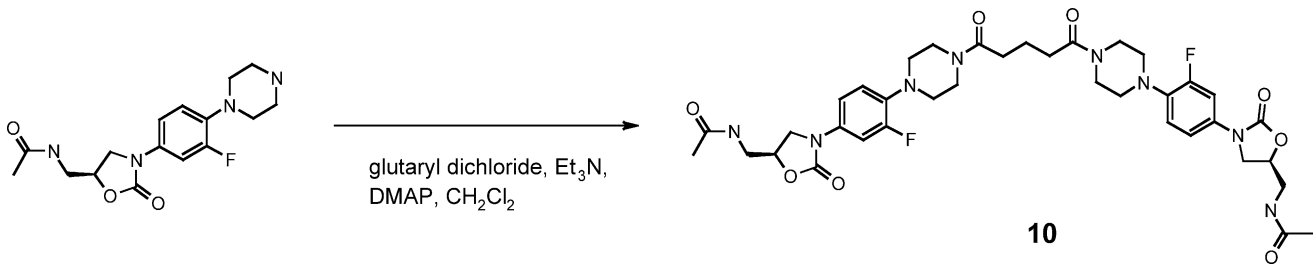

Chart 1.

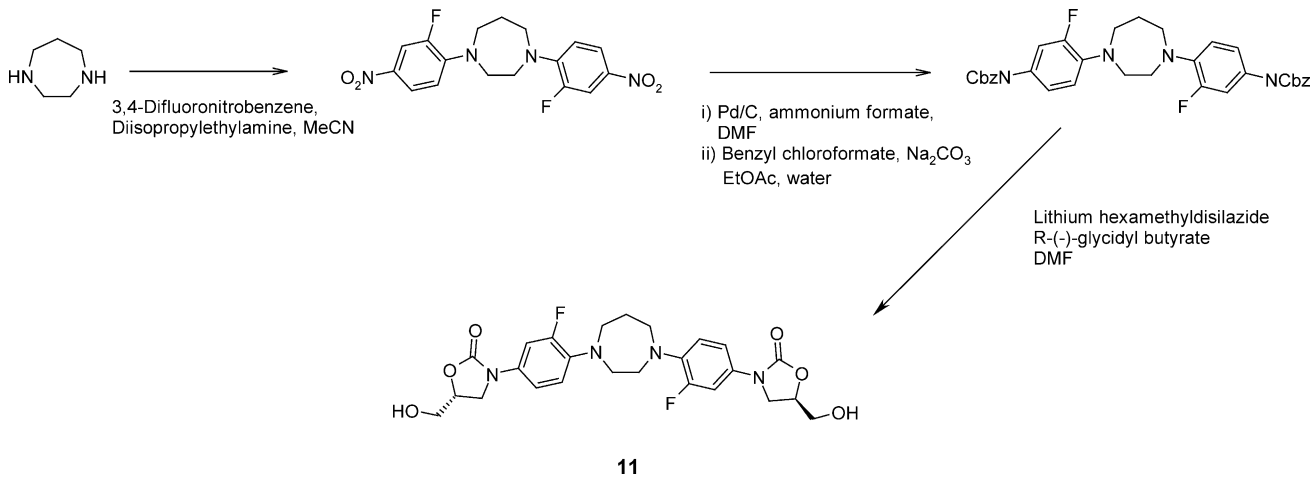

Chart 2.

Triton X-100. K_i values were determined using a substrate range of 0.1–0.9 mM kynuramine [3] or 0.03–1 mM 1-methyl-4-(1-methylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine [24] at six different inhibitor concentrations over a 10-fold range. The formation of the product was followed spectrophotometrically at 314 nm (ε = 12,300 M^{-1} cm^{-1}) or 343 nm (ε = 24,000 M^{-1} cm^{-1}), respectively, and the kinetic constants determined using the Shimadzu software. Data are presented in a Lineweaver–Burke plot to illustrate the unchanged V_{max}.

Spectra were recorded in a Shimadzu UV-2101PC spectrophotometer in an anaerobic cuvette containing MAO A at 10–15 μM in 50 mM potassium phosphate, pH 7.2, containing 0.05% Brij. Inhibitor-induced spectral changes are known to be fast [1], so spectra were recorded as soon as the absorbance had stabilized, approximately 5 min after mixing and repositioning the cuvette. Aliquots of dithionite were then added to the anaerobic enzyme-inhibitor mixture to reduce the flavin. Spectra were recorded 10 min after the additions to allow redox equilibration.

2.3. Molecular modelling

Docking studies of oxazolidinones to the homology model of MAO A were performed using the QXP/FLO software [25]. Residues in the substrate cavity were allowed full conformational flexibility in the docking studies.

3. Results

The lead compound for this work was linezolid **1**, the first oxazolidinone to be approved for use as an antibacterial agent. The chiral C-5 side chain on the oxazolidinone ring was varied as acetamidomethyl, hydroxymethyl, or aminomethyl for each of three aryl substituents on the oxazolidinone ring nitrogen, namely 4-(*N*-morpholino)-phenyl, 4-(4′-bromo-*N*-imidazoyl)-phenyl, and 4-(*N*′-2-(pyrazino)-*N*-piperazinyl)-phenyl (Fig. 1). The *N*-aryl substituents differ in electronic and spacial attributes. The morpholino group is non-aromatic so is not planar and lacks the π electron system of the 4′-bromo-*N*-imidazoyl and *N*′-2-(pyrazino)-*N*-piperazinyl groups. Since an aromatic group favors binding for all MAO ligands, the aromatic, nitrogen-containing groups may favor location of that part of the ligand between the two tyrosines (Y407 and Y444 in MAO A) shown by crystal structures to orient ligands by stacking [20]. Novel compounds **10**–**12** were studied to allow assessment of the orientation of the oxazolidinone molecules in the long channel that forms the active site of MAO A. These are essentially two oxazolidinone molecules linked by aryl substituents ''head-to-head'' or by C-5 side chains ''tail-to-tail'', so that both ends of the molecule are identical. The effects of these compounds on MAO A allow definition of the influence of the acetamidomethyl, hydroxymethyl, and *N*-morpholino groups when bound near the flavin.

The oxazolidinone derivatives used here are all reversible competitive inhibitors of MAO A as shown for linezolid **1** in Fig. 2. The observed inhibition increased slightly with time but was maximal within 2 min. The K_i values for the derivatives were determined from the apparent K_m values at varying inhibitor concentrations.

The K_i values for the compounds vary over a more than 500-fold range (Table 1). In the morpholino series, the hydroxyl derivative **2** is the most effective inhibitor at 1.2 μM, with the amino derivative **3** 100-fold less effective. A similar pattern is seen in the bromo-imidazoyl series where the hydroxyl derivative **5** has a K_i of 0.34 μM and in the pyrazino-piperazinyl series where the K_i for **8** is 0.16 μM. The dimer inhibitors are at least as effective as the monomeric versions, with some improvement for **10** compared to **1** which might be due to the increased interactions of the longer molecule with the large cavity of MAO A.

Table 1
K_i values for the inhibition of MAO A by oxazolidinones and the influence of their binding on the spectrum of MAO A and stabilization of the semiquinone during chemical reduction

Series	Compound	R	K_i (μM)	Spectrum (nm)	Reduction
Morpholino	**1**	Acetamide	20	↑ 510	No SQ
	2	OH	1.2	↓ 495	No SQ
	3	NH_2	116	↓ 495	Substrate
Br-imidazole	**4**	Acetamide	5	Hybrid	Some SQ
	5	OH	0.34	↓ 495	Some SQ
	6	NH_2	23	–	Substrate
Pyrazino-piperazinyl	**7**	Acetamide	1.6	↑ 510	No SQ
	8	OH	0.16	↓ 495	No SQ
	9	NH_2	5.7	–	Substrate
Dimer acetamide	**10**		0.5[a]	↑ 510	Some SQ
Dimer OH	**11**		1[a]	↓ 495	–
Dimer morpholino	**12**		90[a]	–	–

(–) Not determined; SQ, semiquinone.
[a] IC_{50} at 0.15 mM kynuramine. Trial experiments indicated that the dimers give competitive inhibition, so K_i values can be calculated from the relationship $IC_{50} = K_i(1 + S/K_m)$. For the conditions of this table, this means that the K_i values would be: for **10**, 0.25 μM; **11**, 0.5 μM; **12**, 45 μM.

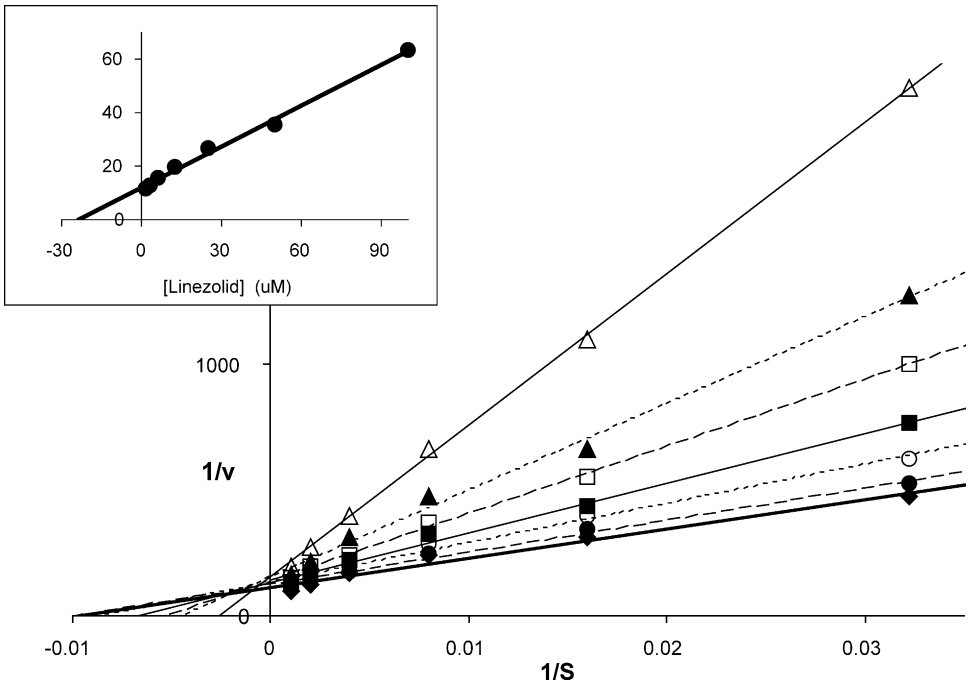

Fig. 2. Linezolid inhibits MAO A competitively: Lineweaver–Burke plot for the inhibition of MAO A by **1**. MAO A activity was measured spectrophotometrically with the substrate 4-(1-methyl-2-pyrryl)-1 methyl-1,2,3,6-tetrahydropyridine (31–1000 μM). The inhibitor **1** concentrations were 0 (◆), 1.56 (●), 3.2 (○), 6.25 (■), 12.5 (□), 25 (▲), and 50 μM (△). The inset shows the secondary plot of the slopes.

However, considering the K_i values for **1**, **10**, and **12** in relation to structure, another explanation is possible. The dimer with acetamide at both ends **10** has an IC_{50} of 0.5 μM but the dimer with morpholino at both ends **12** has an IC_{50} of 90 μM. If the monomer compound **1** with morpholino at one end and acetamide at the other end can bind in either orientation, the IC_{50} obtained would depend on the proportion of enzyme occupied by each mode.

The amino derivatives **3**, **6**, and **9** are poor inhibitors and are also poor substrates that reduce MAO A at relatively slow rates when incubated at 30 °C under anaerobic conditions. The amino derivative in the bromo-imidazole series, **6**, reduces the enzyme within 10 min but the amino derivative in the morpholino series, **3**, requires 4 h to reduce it (see below).

The spectral changes that occur on ligand binding to MAO A are shown in Fig. 3. The slow rate of reduction of MAO A by the amino derivative **3** allowed investigation of the spectral change for substrate binding. The difference spectrum for binding of **3** (Fig. 3, top right), taken before reduction has progressed significantly, shows a minimum at 495 nm similar to that observed for most of the inhibitors previously investigated [2,3]. The hydroxyl derivatives **2**, **5**, **8**, and **11** all produce a difference spectrum with a minimum at 495 nm (Fig. 3, middle column), the same as for the amino derivative. Other features of the difference spectrum are another minimum at 465 nm and a set of triple peaks between 380 and 460 nm where the absorbance is increased compared to the unliganded enzyme.

The spectral changes for derivatives bearing the acetamide group are distinctly different (Fig. 3, left column). These spectra feature a peak at 510 nm in the difference spectrum. This 510 nm peak is also observed with D-amphetamine [7]. Other features of the difference spectra for **1**, **4**, **7**, and **10** include close to zero change at about 480 nm and a double trough between 410 and 450 nm. The difference spectrum for **4** (Fig. 3, left column, second row) is a little different, in that it has a smaller peak at 510 nm and is dragged down below 500 nm. This would be consistent with a small proportion of binding in another orientation that induced the pattern with a 495 nm minimum.

In general, previous studies showed that inhibitors increased the yield of the semiquinone form of MAO A during reduction with dithionite. Both D-amphetamine, which induces a 510 nm increase in absorbance when bound to MAO A, and pirlindole, binding of which results in a decrease in absorbance at 500 nm [7], increased the yield of semiquinone produced by chemical reduction of MAO A, and moreover, prevented full reduction. However, for the reduction of the MAO A–oxazolidinone complexes by dithionite, the 412 nm peak typical of the semiquinone was seen for only a few compounds (Fig. 4). Although compound **1** gave the same difference spectrum on binding as for D-amphetamine, it did not increase the semiquinone peak at 412 nm (Fig. 4, top left) and full reduction followed. As expected, the amino derivative substrates destabilized the semiquinone so that none was seen in the course of the substrate reductions by **3** and **6** (Fig. 4, right). For the remaining inhibitors, different yields of semiquinone are seen with most stabilization by the bromo-imidazole series (**4** and **5**), but in all cases full reduction is possible. For the dimeric inhibitor **10**, the concentration was not high

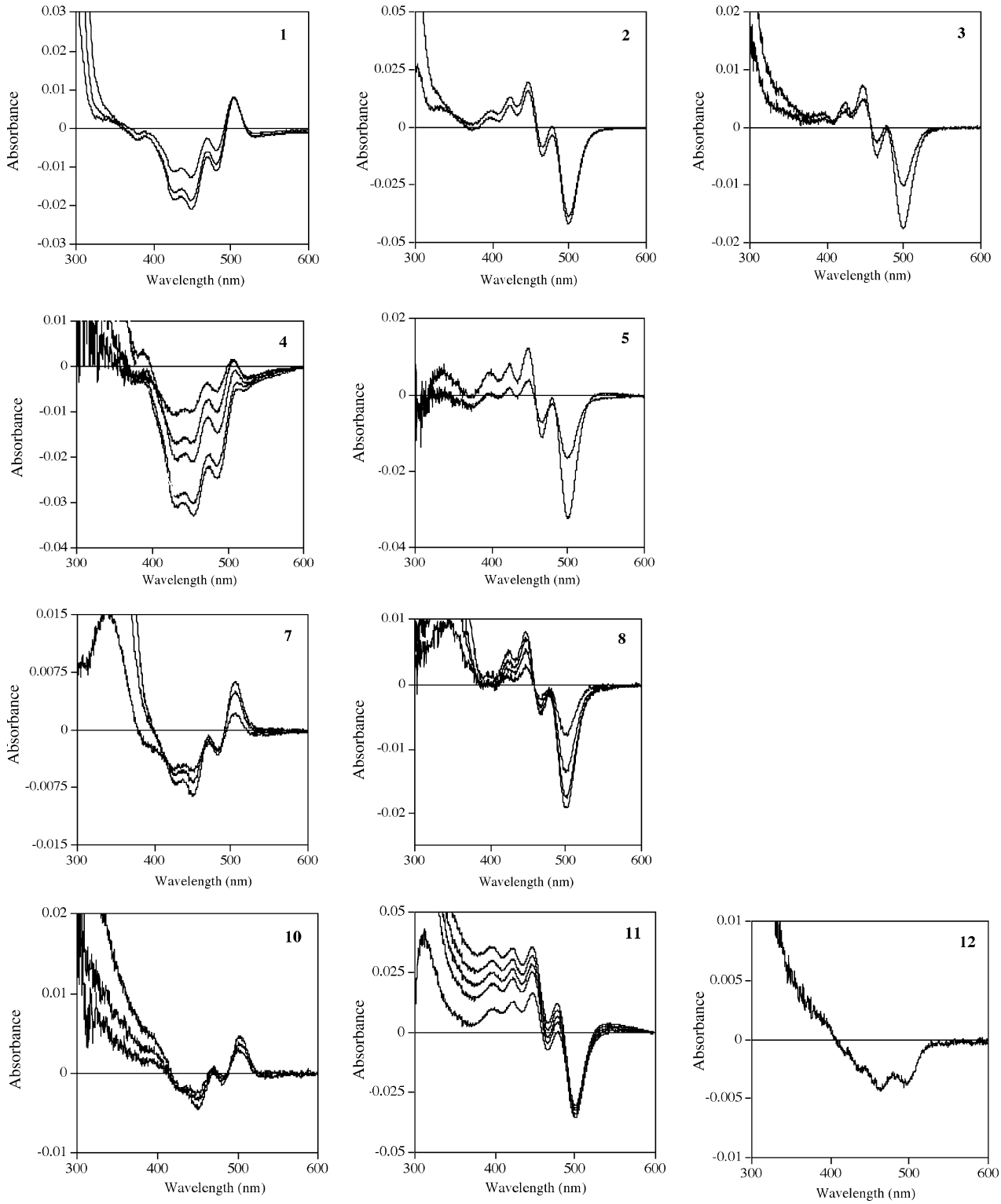

Fig. 3. Ligand-induced spectral changes in MAO A. Difference spectra are shown for the addition of inhibitors as follows: (**1**) 40, 320, and 620 μM **1** to 16 μM MAO A; (**2**) 16 and 160 μM **2** to 14 μM MAO A; (**3**) 4, 6, 60, and 200 μM **3** to 15 μM MAO A; (**4**) 20, 60, 240, 320, and 600 μM **4** to 25 μM MAO A; (**5**) 4 and 40 μM **5** to 12 μM MAO A; (**7**) 4, 12, and 20 μM **7** to 10 μM MAO A; (**8**) 0.5, 1, 1.5, and 2.5 μM **8** to 11 μM MAO A; (**10**) 8, 12, and 36 μM **10** to 9 μM MAO A; (**11**) 8, 24, 32, and 56 μM **11** to 11 μM MAO A; (**12**) 20 μM **12** to 11 μM MAO A.

enough to ensure full saturation due to solubility limitations, so full reduction could occur via free enzyme.

Fig. 5 shows the most favorable binding mode for **1** and **2** identified by QXP. The models of the inhibitors bound to MAO A indicate that for most oxazolidinones, the C-5 substituent on the oxazolidinone ring is furthest into the enzyme cavity, as shown in Fig. 5 for **1** (Fig. 5a) and **2** (Fig. 5b). Each is docked in the active site close to the

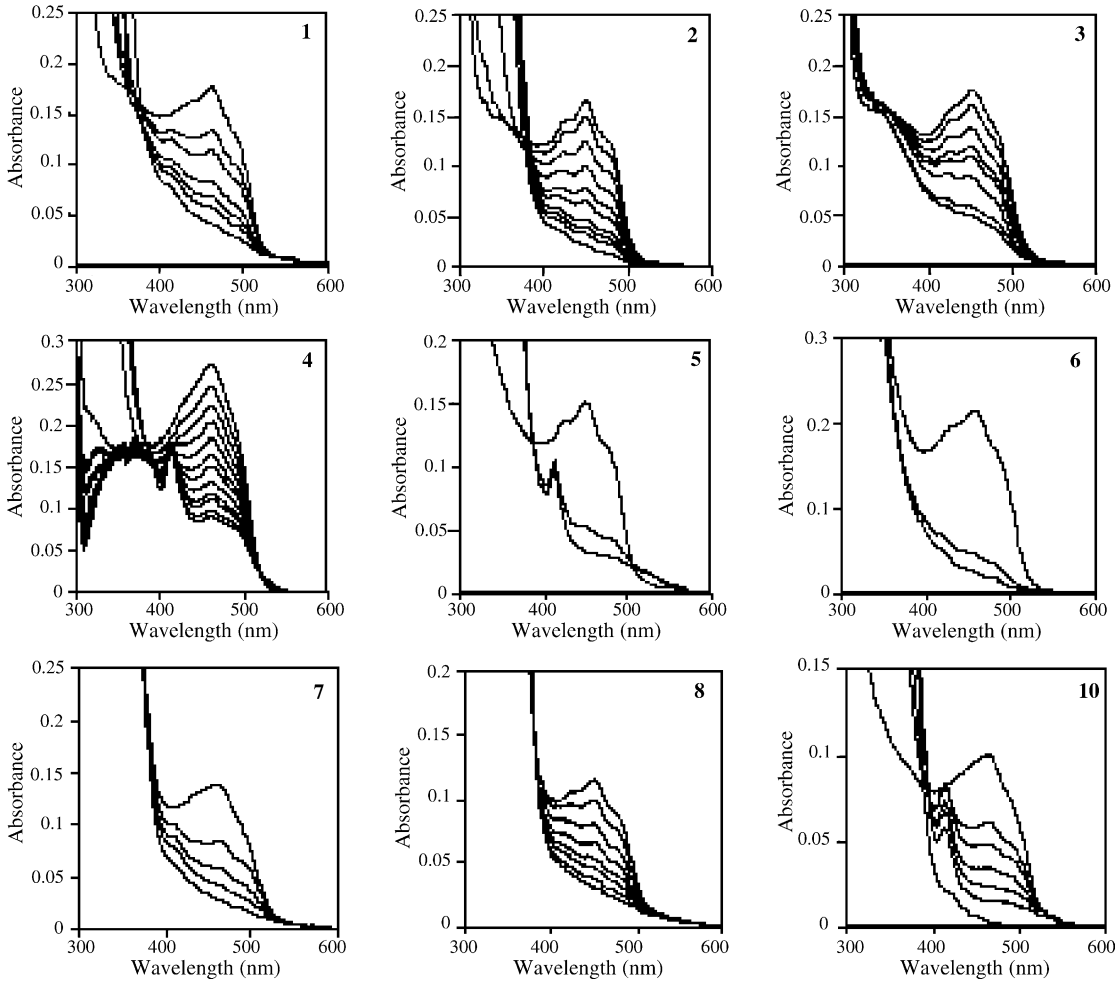

Fig. 4. Spectral changes during reduction of the MAO A–oxazolidinone complexes. Spectra for addition of dithionite to anaerobic MAO A in the presence of inhibitors are shown, except for the substrates **3** and **6** where no reductant was added. Starting from the top left: 16 μM MAO A with 3.6 mM (**1**), 14 μM MAO A with 0.66 mM (**2**), 15 μM MAO A with 0.2 mM (**3**) where spectra were recorded over 4 h, 25 μM MAO A with 2 mM (**4**), 12 μM MAO A with 0.56 mM (**5**), 17 μM MAO A with 0.1 mM (**6**) where spectra were recorded at 10 and 20 min, 9 μM MAO A with 0.2 mM (**7**), 11 μM MAO A with 80 μM (**8**), and 13 μM MAO A with 0.284 mM (**10**). (Note that the bottom right spectral picture has been placed for compact presentation. The bottom right panel shows the spectra for compound **10** which has two acetamide end groups and so it should be compared with the other spectra in the left column of this figure.)

flavin (pink) and between tyrosines 407 and 444 (green). Hydrogen bonding interactions for these inhibitors with the active site residues are indicated by dashed yellow lines. Linezolid **1** forms two hydrogen bonds to tyrosine 197 and to glutamine 215. Compound **4** gave a similar picture to **1** with the acetamide group between the tyrosines 407 and 444 as shown in Fig. 5a. However, with **4**, an alternative possible binding mode was also identified (Fig. 5c). In this alternative binding mode, the bromo-imidazole ring is furthest into the cavity and occupies a position close to the flavin similar to the oxazolidinone ring in **2**.

The models of binding for **1** and **2** show significantly different interactions with the two orienting tyrosines 407 and 444. With **2** the oxazolidinone ring stacks with the tyrosines as expected for a substrate [26] whereas with **1** the acetamide lies between the tyrosines providing no π-stacking interactions. It is possible that the spectral differences between the acetamide and OH series seen in Fig. 3 could be due to altered effects on the flavin arising from the difference in the interactions with the substrate-orienting tyrosines.

Two energetically similar binding modes were also found for the substrate **3** as shown in Fig. 6. One (Fig. 6b) is similar to the result obtained for the hydroxyl derivative **2** (Fig. 5b) with the same three hydrogen bonds to asparagine 181, tyrosine 197, and the backbone carbonyl of glycine 443. In the other mode (Fig. 6a), the amine is within van der Waals distance of the N-5 of the flavin. This position is the one likely to allow the observed slow reduction of the flavin.

4. Discussion

This study has probed the orientation of the oxazolidinone analogues in the active site of MAO A. Comparison of the K_i values (Table 1) for the dimeric compounds, **10** and **12**, with the monomer **1** that has an acetamide at one end

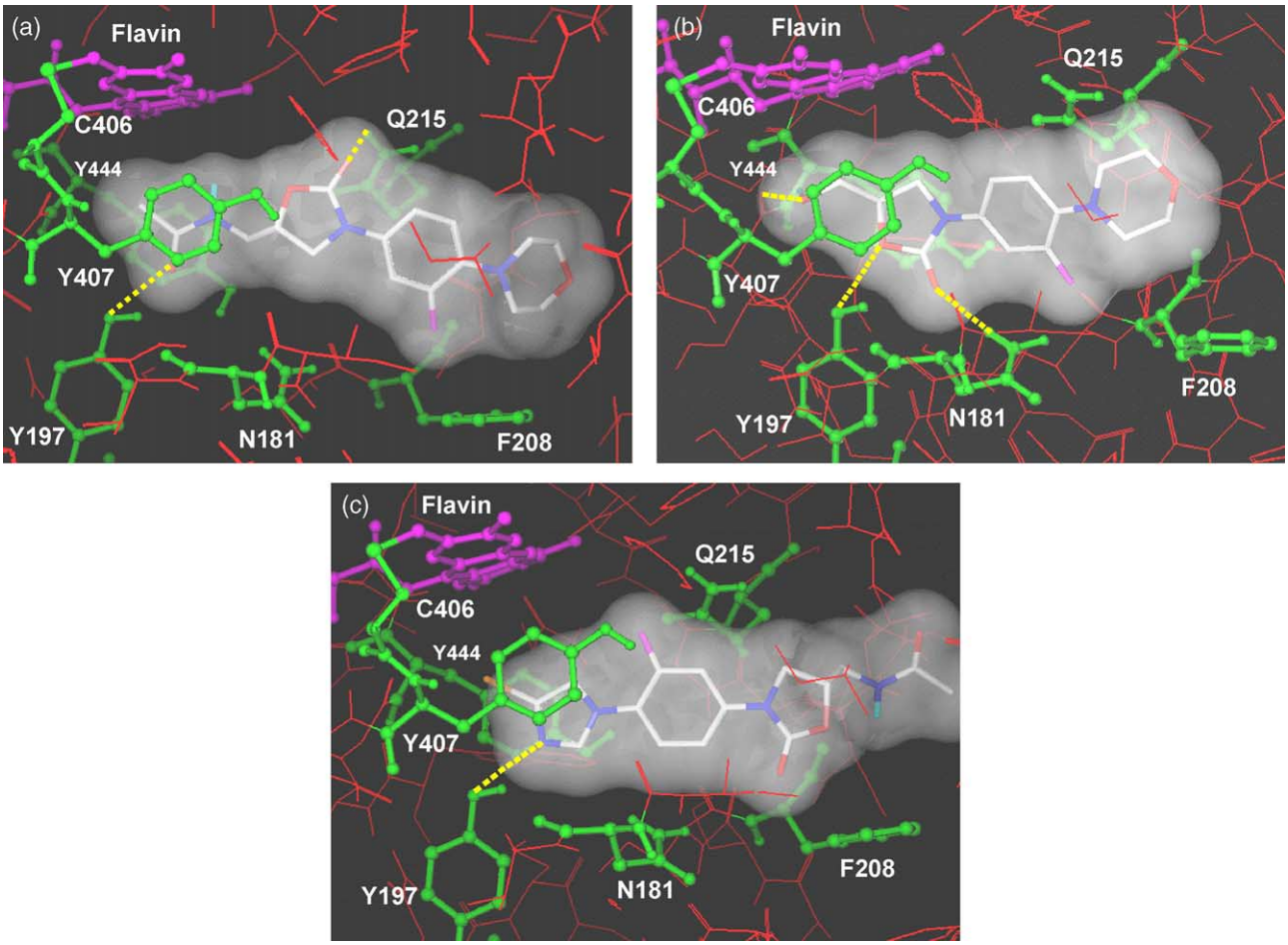

Fig. 5. Modelled positions of oxazolidinone derivatives with respect to the FAD in the active site of MAO A for (a) compound **1**, (b) compound **2**, and (c) compound **4.** The oxazolidinones are docked in the active site close to the flavin (pink) and between tyrosines 407 and 444 (green). Dotted yellow lines indicate hydrogen bonds. Models of inhibitor-liganded MAO A indicate that with most oxazolidinones the substituent on the five-membered oxazolidinone ring is furthest in to the enzyme cavity. However, with **4**, an alternative binding mode is seen. The bromo-imidazole ring is able to occupy a position close to the flavin similar to the oxazolidinone ring in **2**.

and morpholino at the other suggested that **1** binds to MAO A preferably in the orientation that places the acetamide near the flavin. The spectral change induced by binding of **10** (acetamide–acetamide) to MAO A shows a difference spectrum with the 510 nm increase and very little decrease between 440 and 500 nm. However, the compound **12** (morpholino–morpholino) gave a clear decrease at 495 nm and no 510 nm peak. The spectra for **1** (morpholino–acetamide) suggest that the acetamide binding close to the flavin is the favored mode, consistent with the K_i values of **10** and **11** and with the optimum docking shown in Fig. 5a. For the bromo-imidazoyl-acetamide compound **4**, the spectra (Fig. 3) are clearly a hybrid of the two types, suggesting that binding with either end entering the active site cavity first is possible for this compound. The modelling study found two binding modes for **4**, one with acetamide near the flavin (as shown for **1** in Fig. 5) and one with the bromo-imidazole group inwards (Fig. 5c), consistent with these experimental results.

The end of the molecule entering first should bind closest to the N-5 of the flavin where the electron transfer must take place for substrates. The data here are consistent with what is known about binding from the crystal structure of MAO B, showing that two tyrosine residues align the molecule for optimum orientation towards the N-5 of the flavin [20]. Ab initio molecular orbital calculations for other oxazolidinone derivatives have shown that the electronic properties of the oxazolidinone ring are good for stacking with aromatic systems [27].

The docking of energy-minimised models of the oxazolidinone compounds to the structure of MAO A [4] was performed to seek an explanation for the very clear spectral difference between an acetamide group near the flavin (left column of Fig. 3) or an amino (right), or hydroxyl (middle column of Fig. 3) group near it. The modelled positions are shown in Fig. 5. For **2** and **4**, stacking of the oxazolidinone and imidazole rings between the tyrosines is consistent with what has been seen in the crystal structures of MAO B [26]. For **1**, the acetamide group shifts the oxazolidinone ring away from the tyrosines and the acetamide nitrogen is seen there instead (Fig. 5a). The amino derivative **3** in Fig. 6 can either take a position like **2** with ring overlap

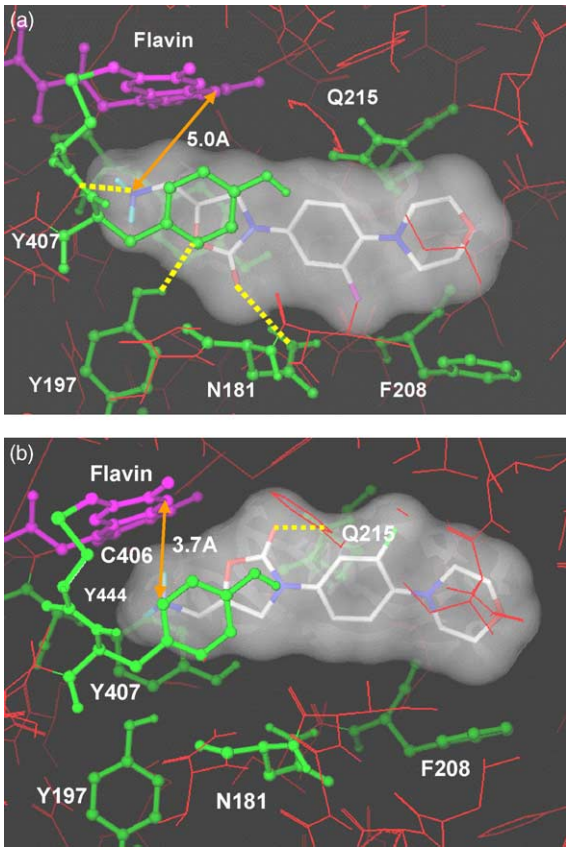

Fig. 6. (a and b) Alternative modelled positions of **3** with respect to the FAD in the active site of MAO A. The distance between N-5 of the flavin and the amine is indicated.

with the tyrosines or one where the amine is in the correct position for electron transfer to the flavin.

The models do not provide any obvious explanation for the influence of inhibitors on the redox properties of the flavin. The inhibitors used in previous studies, such as D-amphetamine and pirlindole, prevented full reduction of the flavin so that the semiquinone state indicated by the 385 and 412 nm peaks in the difference spectra for dithionite titrations of MAO A was maximised [7]. Dithionite titrations of MAO A in the presence of the oxazolidinone inhibitors (Fig. 4) either resemble that seen with free enzyme with an estimated yield of about 35% of the maximum semiquinone [2] or they resemble substrate titrations (Fig. 4, right) which show no semiquinone at all. This could be interpreted as these molecules having a relatively non-specific fit in the active site, blocking substrate access but not staying sufficiently close to the flavin to prevent its full reduction. It is interesting that another oxazolidinone, befloxatone, an antidepressant devoid of antibacterial activity, also gave a substrate-like reduction pattern with no trace of the 412 nm peak [2].

Taken together the theoretical and experimental results give insight into the improved potency of the hydroxymethyl derivatives over the acetamides. Fig. 5 shows that the compounds **1** and **2** take up different binding poses in the active site, with the oxazolidinone ring carbonyl pointing up in Fig. 5a but down in Fig. 5b. The hydroxyl group of **2** forms a hydrogen bond to a backbone residue pulling the ligand well into the hydrophobic binding pocket so that the middle ring is under Q215 in **2**. In contrast, with **1** it is the oxazolidinone ring carbonyl that H-bonds to Q215. The better inhibitor (**2**, with the OH) also has three hydrogen bonds whereas only two bonds are formed with **1**. Both of these factors could contribute to the higher affinity observed for the hydroxymethyl compounds. The apparent lower affinity for the aminomethyl derivatives compared to the hydroxymethyl compounds that model similarly into the active site is simple: the amino group pK_a is likely to be around 9–10 so only a small proportion will be in the neutral form that binds to MAO.

The models, difference spectra, and the semiquinone stabilization all indicate that bromo-imidazole can bind towards the flavin but there is no such indication for the pyrazinyl piperazino compounds **7** and **8**. The pyrazinyl piperazino group is somewhat similar to the bromo-imidazole but clearly differs from the morpholino in having the extra heteroaromatic ring. The preferred binding of such lipophilic aromatic residues over cycloaliphatic and more polar rings is well documented in the early literature on MAO A and MAO B inhibitor structure–activity relationships. Such interactions may be based on π-stacking interactions with aromatic amino acids (see references quoted in [14]) in an area of the cleft that influences A/B specificity [4,28].

5. Conclusions

Modelling studies reveal alternate binding modes for oxazolidinones in the active site of MAO A. Experimental support for aryl group binding near the flavin comes from the substrate behaviour of the amino derivatives and favored binding of dimers with aryl groups at both ends (compounds **10** and **11**). When only the bulky morpholino group is available as in the dimer **12**, the molecule is a poor inhibitor. Placement of the bromo-imidazole group near the flavin was found as an alternate binding mode and its presence there was supported by the mixed spectral patterns obtained for **4**. In general, efficacy of inhibition of MAO A was favored by binding of the oxazolidinone ring in the aromatic cage close to the flavin, reinforced by hydrogen bonding.

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