Facile, Expeditious and Cost-effective Preparation of N-Phthaloyl (S)-Amino Acids and Their in silico Activities against Staphylococcus Aureus

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Exploration of cost-effective and eco-friendly synthetic processes that offer high yields is a trending issue these days among organic chemists. Selecting suitable protecting groups is a crucial step in this exploration. In this context, N-phthaloyl amino acids are significant intermediates in a variety of chemical and biological processes. The ease of addition and removal of the phthaloyl moiety makes it ideal for masking the primary amino group, without the complications of side reactions associated with the strong nucleophilicity of the nitrogen atom in its unmasked state. In the literature, a number of synthetic routes have been reported for N-protection of amino groups by using phthalic anhydride/acid (Table 1). The N-protection of (S)-amino acids with phthalic anhydride/acid at high temperatures leads to racemization, and organic solvents with high dielectric constants are required to facilitate masking under mild reaction conditions. Thus the phthaloyl N-protection of achiral amines was carried out at high temperature and pressure by Fraga-Dubreuil and co-workers, and aromatic solvents or catalysts were used in other studies (Table 1, entry 1-5). The N-phthaloylation of chiral (S)-amino groups could be performed at reduced pressure or in the presence of organic solvents and catalysts (Table 1, entry 7-9).

Phthalimides themselves have a broad spectrum of biological activities, including antibacterial and anticancer properties. They have been extensively used in the synthesis of fluoroprobes, DNA cutting tools, optical brighteners and dyes. Antimycobacterial derivatives of phthalimide have been prepared in several studies. The chemical nature of the phthalimide functionality (-CO-N(R)-CO-) is predominantly hydrophobic, which may facilitate their ability to cross biological barriers.

Green chemistry emphasizes environmentally friendly and efficient processes of synthesis. Key components of green chemistry approaches are the use of solvent-free conditions or natural catalysts, rather than using volatile, hazardous and costly organic
The fruit juice of citrus lemon is an effective natural catalyst, containing mainly water, carbohydrates, ascorbic acid and citric acid. Lemon juice is about 3-7% citric acid, with pH between 2 and 3. Microwave assisted (MW, \( m \)) reactions support the goals of green chemistry because they generally are high yielding in short reaction times and have been demonstrated to facilitate synthesis under solvent-free reaction conditions.10

In the present work, we introduce a green high-yielding approach for the N-phthaloylation of (S)-amino acids (Scheme 1) and make note of the fact that the optical rotations of products 3a-f show good agreement with literature values, indicating that there is no racemization.

In optimizing our procedure, we investigated the N-protection of (S)-amino acids (1a-f) under a variety of experimental conditions, and the results are shown in Table 2. In each case, we were guided by the practical concerns of achieving the best yields and lowest racemization and made no attempt to examine every permutation of conditions. Under MW, when the power of the microwave was tuned to medium high (500-800W), the reaction tended to completion within a few minutes with excellent yield (Entry 2, 95-98%). Higher MW power led to decomposition, and lower MW power gave incomplete conversion. Reactions with conventional heating were also explored as an alternative method, bearing in mind the precedent for keeping the temperature below 135 °C, as high temperatures will lead to racemization.5 At 130-135 °C, excellent (95-98%) yields were achieved in 15 minutes (Table 2, entry 9). Under lemon juice catalysis, N-protection did not occur under ambient conditions, not even with overnight stirring or grinding the reactants (Table 2, entries 10-11). The N-phthaloylation of chiral amino acids in the presence of acetic acid was also tried under these conditions (Table 2, entries 5-7), but yields and reaction times were less favorable.

As it is the most significant organic acid in lemon juice (3-7%), we assume that citric acid is responsible for catalyzing the entire N-protection reaction. To investigate the likely participation of this triprotic acid during the reaction (Scheme 2), we carried out our procedure in the presence of an aqueous solution of commercially available citric acid at the same concentration and volume. The results obtained from commercial citric acid were found to be consistent with those obtained from natural juice (Table 2, entries 7 and 13).

Table 1. Synthetic methods of N-Phthalimide under different reaction conditions.

| Entry | Substrate | Eq \(^a\) | T(°C) | Time (min) | Catalyst | Solvent | Yield (%) | Technique |
|-------|-----------|----------|-------|------------|----------|---------|-----------|-----------|
| N-protection of simple amine (RNH\(_2\)/racemic amino acids (AA)) |
| 1     | RNH\(_2\), AA | 1 – | 15-20 | AcOH | – | Aromatic solvents | 81-100 | MW, Δ \(^{14}\) |
| 2     | RNH\(_2\) | 1 – | 2-10 | TEA | AcOH /toluene | 90 | Δ \(^{1}\) |
| 3     | AA | 1 reflux | 120 | – | H\(_2\)O, EtOH | 76-86 | Δ \(^9\) |
| 4     | RNH\(_2\), AA | 1 reflux | 150 | TEA | Aromatic solvents | 50-90 | Δ \(^{10}\) |
| 5     | RNH\(_2\), AA | 1 reflux | 170 | 20 resins | DMF | 13-78 | MW \(^{16}\) |
| N-protection of asymmetric amino acids (S-configuration) |
| 7     | (S)-AA | 1.5 | 130-150 | 30-60 | – | – | 79-99 | Δ \(^{16}\) |
| 8     | (S)-AA | 1 – | 2 | DMF | 4-DMAP/TEA | 30-96 | MW \(^{15}\) |
| 9     | (S)-AA | 1 reflux | 1440 | TEA | THF | 97 | Δ \(^{11}\) |
| 10    | (S)-AA | 1.1 – | 1.5-12 | L\(^2\) | – | 95-98 | MW \(^*\) |
| 11    | (S)-AA | 1.3 | 130-135 | 15 | L\(^2\) | 97-98 | Δ \(^*\) |

\(^{a}\)eq of phthalic anhydride for 1 eq of substrate; \(^{\dagger}\)heating under high pressure; \(^{\ddagger}\)heating under reduced pressure; \(^*\)our method; \(^{\dagger}\)lemon juice.
The specific rotation of the $N$-protected derivatives 3a-f, obtained from MW and conventional heating was found consistent with the literature values (Table 3). These findings support the conclusion that ($S$)-amino acids did not suffer racemization under either set of conditions.

Other spectroscopic techniques provided consistent information towards the structures of chiral phthalides 3a-f. This was indicated by the appearance of IR signals for C=O (both acid and amide) and OH groups near at 1730 and 3470 cm$^{-1}$, respectively, and by $\lambda_{\text{max}}$, in the absorption spectrum near 312 nm.

**Scheme 1.** Formation of $N$-phthaloyl $S$-amino acids.

| Entry | Catalysts | Time, min | $T$ ($^\circ$C) | Technique | Power level | Yield % |
|-------|-----------|-----------|----------------|-----------|-------------|---------|
| 1     | Lemon juice $^a$ | 2-10      | –              | MW        | High        | Low yield |
| 2     | Lemon juice $^b$ | 1.5-12    | –              | MW        | Medium high | 95-98   |
| 3     | Lemon juice $^b$ | 3-15      | –              | MW        | Medium      | 34-60   |
| 4     | Lemon juice $^c$ | 30        | –              | MW        | Low         | –       |
| 5     | Acetic acid $^d$ | 13        | –              | MW        | Medium high | 98      |
| 6     | Acetic acid $^e$ | 15-20     | –              | MW        | Medium      | 65-78   |
| 7     | Acetic acid $^e$ | 3-13.5    | –              | MW        | Medium high | 79-90   |
| 8     | Lemon juice $^a$ | 20-30     | 130            | Heat      | –           | 80-89   |
| 9     | Lemon juice $^a$ | 30        | 110-120        | Heat      | –           | 65-70   |
| 10    | Lemon juice $^a$ | 15        | 130-135        | Heat      | –           | 97-98   |
| 11    | Lemon juice $^a$ | 30        | ambient        | grinding  | –           | –       |
| 12    | Citric acid $^f$ | 14        | ambient        | stirring  | –           | –       |

$^a$0.1 mL, $^b$1.5 mL, $^c$high = 800-1000 W, medium high = 500-800, medium = 400-500 W, low = 100-400 W, $^d$0.1 mL of 7% aq. solution is used for the $N$-protection of ($S$)-leucine.

**Scheme 2.** Possible mechanism of formation of $N$-phthaloyl $S$-amino acids.
The splitting patterns of 1H-NMR of all N-protected derivatives showed a multiplet of four aromatic protons at approx. δH 7.80-7.90 ppm belong to isoindoline aromatic cycle. The aliphatic H signals of 3a and 3b were observed as doublets at δH 1.65 and 0.88/1.14 ppm, respectively. The doublets at δH 0.92 (J = 6.8 Hz) and 0.94 (J = 6.8 Hz) ppm, multiplets at δH 1.46, 1.93 and 2.31 ppm in the NMR of 3c indicated the presence of the isobutyl group. The methylthio protons of 3d gave singlet signal at δH 2.04 ppm. The 1H NMR spectra of compounds 3e and 3f showed aromatic doublet and multiplet signals in the range of δH 6.95-7.60 ppm. The LR-EIMS of the N-phthaloyl (S)-amino acids showed the molecular ion peak [M]+/C19.

Compounds 3b-d contain γ-hydrogens and undergo Mc-Lafferty rearrangement, therefore revealing an intense peak at m/z = 205 amu.

To probe the potential antibacterial activities of 3a-f, we used in silico methods. In the field of structure based drug design, the assessment of ligand-receptor complexes is very helpful.33,34 We compared the structure and stereochemistry of 3a-f with sulbactam (Figure 1), a Class A β-lactamase inhibitor. The relevant chiral carbon (C-2 in both cases) has the S configuration, making sulbactam an appropriate choice for comparative docking studies with 3a-f. We performed docking studies of these chiral molecules inside the active cavity of the β-lactamase of Staphylococcus aureus.

Table 3. Reported and calculated values of specific rotation, yield and spectroscopic data of 3a-f.

| Entry | Reported [α]20 D | Observed [α]20 D | Yield (%) | ω (cm⁻¹) | λmax, nm<sup>c</sup> |
|-------|----------------|-----------------|-----------|---------|----------------------|
| 3a    | −23.0<sup>d</sup> | −23.7           | 98        | 3477    | 20504 (296)         |
| 3b    | −68.3<sup>d</sup> | −68.5           | 97        | 3489    | 1732                 |
| 3c    | −24.0<sup>17</sup> | −28.5           | 95        | 3483    | 1718                 |
| 3d    | −46.1<sup>d</sup> | −44.0           | 96        | 3462    | 1728                 |
| 3e    | −249.6<sup>d</sup> | −249.0          | 95        | 3397    | 1711                 |
| 3f    | −215.7<sup>d</sup> | −214.0          | 95        | 3271    | 1771                 |

<sup>a</sup>Power level is medium high; <sup>b</sup>at 130-135 °C; <sup>c</sup>in EtOAc.

Table 4. The 1H NMR splitting pattern of asymmetric and diastereotopic protons of 3a-f.

|   | δ ppm (J, Hz) |
|---|---------------|
| 3a|               |
| 3b|               |
| 3c|               |
| 3d|               |
| 3e|               |
| 3f|               |

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Sulbactam inhibits the $\beta$-lactamase by making a bond with the SER70 residue.\textsuperscript{35–41}

Preliminary molecular docking experiments of all compounds gave interesting results (Table 5). The standard drug, sulbactam, with binding energy $-5.6 \text{kcal.mol}^{-1}$, docked with the target through hydrogen bonding with SER70 and the GLN237 residue at a distance of 3.29Å from SER70. It was observed that all the title compounds, except 3f, exhibited hydrogen bonding with SER70 of the active site, with binding energies (BE) less than that of sulbactam. Compound 3a exhibited BE $-5.8 \text{kcal.mol}^{-1}$, docked with residues SER70, ASN132, GLU166 and GLN237 through hydrogen bonding. This binding energy is comparable with that of sulbactam. Modifying the R-chain as shown in structures 3b-3d further reduces the BE value. In addition to H-bond interactions, the compounds 3b-3d also displayed $\pi$-alkyl interactions (Figures 2-3). The modifications shown in compounds 3e and 3f further decrease the binding energy. The compound 3e showed the highest docking energy ($-6.9 \text{kcal.mol}^{-1}$) and displayed four hydrogen bonding interactions with SER70, SER130, ASN170 and SER235. Finally, the molecule 3f showed good docking energy ($-6.8 \text{kcal.mol}^{-1}$) with four hydrogen bonds, but no interaction was observed with the SER70 residue. The results are generally supportive of the idea that the title compounds are predicted to have antibacterial activity.

In conclusion, we have developed a simple and green approach for the protection of (S)-amino acids under solvent free conditions. We found that (S)-amino acids do not undergo racemization in the protection reactions. The specific rotation values and characterization data by UV-VIS, IR, $^1$H-NMR and LR-EIMS of 3a-f were consistent with the previously reported values, confirming that the reaction occurs with retention of configuration. Comparative molecular docking assessments of 3a-f and sulbactam inside the active cavity of the $\beta$-lactamase of \textit{Staphylococcus aureus} (PDB ID: 3BLM) suggest that these compounds may be potential $\beta$-lactamase inhibitors and thus have antibacterial activities against \textit{S. aureus}.

\textbf{Experimental section}

All chemicals were purchased from Sigma Aldrich Chemical Co. Melting points were determined \textit{via} the open capillary method and are uncorrected. Thin layer chromatography (TLC) was performed to check the extent of reaction in which silica gel 60 $F_{254}$ and EtOAc or EtOH were used as stationary and mobile phases respectively. Ninhydrin was used as luminous indicator. An AP-300 Polarimeter was used for determination of specific rotations. A Prestige 21 Spectrophotometer was used to record the IR spectra. A MAT 312 instrument was used to record LR EIMS. Proton NMR spectra were recorded.
Table 5. Molecular docking results for the screened compounds and 3BLM.

| Compound | R group* | BE (Kcal.mol⁻¹) | The residues attached via hydrogen bond | Bond length (Å) with SER70 residue |
|----------|----------|-----------------|----------------------------------------|----------------------------------|
| 3a       | -CH₃     | -5.8            | SER70, ASN132, GLU166, GLN237         | 3.06                             |
| 3b       |          | -6.2            | SER70, ASN132                          | 2.85                             |
| 3c       |          | -6.5            | SER70, ASN132, ASN170                  | 2.34                             |
| 3d       | -(CH₂)₂SMe | -6.3            | SER70, LYS73, SER130, ASN132, ASN170  | 3.19                             |
| 3e       |          | -6.9            | SER70, SER130, ASN170, SER235         | 2.52                             |
| 3f       |          | -6.8            | LYS73, SER130, GLN237                 | –                                |
| Sulbactam|          | -5.6            | SER70, GLN237                          | 3.29                             |

*the substituent directly attached to the chiral carbon atom.

Figure 2. 3D representations of 3BLM-ligands (3a-f) complexes.
on a Bruker (400 MHz) spectrometer. For microwave irradiation, a Dawlance DW-MD4N instrument was used (1000 W). For catalyst preparation, fresh lemons were cut and pressed to obtain the juice. The crude juice was successively filtered through muslin cloth and filter paper. The clear filtrate (having pH 3.2-3.5) was used as the catalyst.

**General procedure for synthesis of (S)-N-phthaloyl amino acids (3a-f)**

**Method A (microwave mediated reaction)**

A mixture of phthalic anhydride (1.1 eq) and the appropriate (S)-amino acid (0.10 g) in a small amount of lemon juice (1-2 drops, 0.1 mL) was irradiated for 5-12 min in a closed flask, the time being signified by TLC in EtOAc or EtOH. The cold reaction mixture was dissolved in methanol, filtered and the residue recrystallized from a mixture of methanol and water (7:3 ratio) to furnish pure crystals of 3a-f.

**Method B (conventional heating method)**

A mixture of the appropriate (S)-amino acid (0.10 g) and phthalic anhydride (1.3 eq) in the presence of lemon juice (1-2 drops, 0.1 mL) was heated at 130-135°C in an oil bath for 15-20 min, as indicated by TLC in EtOAc or EtOH. The cold reaction mixture was dissolved in methanol, filtered and the residue recrystallized from a mixture of methanol and water (7:3 ratio) to furnish pure crystals of 3a-f.

**(2S)-2-(1,3-Dioxoisindol-2-yl)propanoic acid (3a)**

Colorless prisms, yield (%) 97 (conventional heating), 98 (microwave), Mp: 152°C (Lit² 149-151°C), Rf = 0.31 (EtOAc); [x]²⁰_D = -23.7 for MW and -23.2 for conventional heating (c 1.5, EtOH), lit² [x]²⁰_D = -23.0, c 0.8, EtOH; log ε (λ<sub>max</sub>, nm) 3.2003 (296); FT-IR (KBr, ν<sub>max</sub>, cm⁻¹) 3477 (broad s, O–H), 1720 (broad s, C=O); ¹H NMR (400 MHz,
CD$_3$OD, ppm): $\delta_H$ 1.65 (d, $J=7.2$ Hz, 3H), 4.94 (q, $J=7.2$ Hz, 1H), 7.80–7.91 (m, 4H, aromatic region); $^{13}$C NMR (100 MHz, CD$_3$OD, ppm): $\delta_C$ 23.9, 52.5, 122.4, 128.6, 134.9, 168.1, 173.4: LR EIMS (m/z, amu): 219 [M]$^+$ (21%), 175 [M – CO$_2$]$^{++}$ (86%) and 174 [M – $^*$/OH – CO]$^+$ (100%).

(2S)-2-(1,3-Dioxoisooindolin-2-yl)-3-methylbutanoic acid (3b)

White needles, yield (%): 97 (conventional heating), 97 (microwave), Mp: 120 °C (Lit$^2$ 120-122 °C), $R_f = 0.56$ (EtOAc); $[\alpha]^{20}$D: $-68.5$ for MW and $-68.7$ for conventional heating method (c 1.5, EtOH), (Lit$^2$ $[\alpha]^{20}$D $-68.3$, c 1.0, EtOH); log $\varepsilon$ ($\lambda_{max}$, nm): 3.3453 (300); FT-IR (KBr, $\bar{\nu}_{max}$/cm$^{-1}$): 3489 (broad s, O–H), 1732 (broad s, C=O); $^1$H NMR (400 MHz, CD$_3$OD, ppm): $\delta_H$ 0.88 (d, $J=6.8$ Hz, 3H), 1.14 (d, $J=6.4$ Hz, 3H), 2.69 (m, $J=6.8$ Hz, 1H), 4.52 (d, $J=8.0$ Hz, 1H), 7.82–7.89 (m, 4H, aromatic region); $^{13}$C NMR (100 MHz, CD$_3$OD, ppm): $\delta_C$ 19.5, 21.2, 28.3, 57.4, 123.9, 131.5, 133.9, 167.2, 173.6; LR EIMS (m/z, amu): 247 [M]$^+$ (13%), 205 [M – propene]$^+$ (14%), 204 [M – iPr]$^+$ (10%), 203 [M – CO$_2$, A]$^+$ (34%), 202 [M – OH – CO]$^+$ (100%) and 160 [A – iPr]$^+$ (35%).

(2S)-2-(1,3-Dioxoisooindolin-2-yl)-4-methylpentanoic acid (3c)

White needles, yield (%): 98 (conventional heating), 95 (microwave), Mp: 122 °C (Lit$^2$ 120-122 °C), $R_f = 0.50$ (EtOAc); $[\alpha]^{20}$D: $-28.5$ for MW and $-28.4$ for conventional heating method (c 1.5, MeOH), (Lit$^2$ $[\alpha]^{20}$D $-24.0$, c 2.6 in EtOH); log $\varepsilon$ ($\lambda_{max}$, nm): 3.2357 (253); FT-IR (KBr, $\bar{\nu}_{max}$/cm$^{-1}$): 3483 (O–H), 1718 (C=O); $^1$H NMR (400 MHz, CD$_3$OD, ppm): $\delta_H$ 0.92 (d, $J=6.8$ Hz, 3H), 0.94 (d, $J=6.8$ Hz, 3H), 1.44–1.48 (m, 1H), 1.93 (ddd, $J=6.8$ Hz, $J=10.0$ Hz, $J=4.4$ Hz, 1H), 2.31 (ddd, $J=14.2$, $J=11.4$, $J=4.4$ Hz, 1H), 4.90 (dd, $J=11.6$, $J=4.4$ Hz, 1H), 7.81–7.89 (m, Ph H); $^{13}$C NMR (100 MHz, CD$_3$OD, ppm): $\delta_C$ $\delta$ 10.7, 16.5, 26.1, 34.8, 57.4, 124.0, 131.1, 134.7, 168.4, 174.3; LR EIMS (m/z, amu): 261 [M]$^*$ (13%), 205 [M – propene]$^*$ (14%), 204 [M – iPr]$^*$ (10%), 203 [M – CO$_2$, A]$^*$ (34%), 202 [M – OH – CO]$^*$ (100%) and 160 [A – iPr]$^*$ (35%).

(2S)-2-(1,3-Dioxoisooindolin-2-yl)-4-(Methylthio)butanoic acid (3d)

Colorless prisms, yield (%): 97 (conventional heating), 96 (microwave), Mp: 132 °C (Lit$^2$ 129-131 °C), $R_f = 0.69$ (EtOH); $[\alpha]^{20}$D: $-44.0$ for MW and $-45.8$ for conventional heating method (c 1.5, MeOH), (Lit$^2$ $[\alpha]^{20}$D $-46.1$, c 1.16, EtOH); log $\varepsilon$ ($\lambda_{max}$, nm): 3.2390 (298); FT-IR (KBr, $\bar{\nu}_{max}$/cm$^{-1}$): 3462 (O–H), 1728 (C=O); $^1$H NMR (400 MHz, CD$_3$OD, ppm): $\delta_H$ 2.04 (s, 3H, SCH$_3$), 2.47–2.58 (m, 4H), 5.10 (t, $J=7.2$ Hz, 1H) and 7.81–7.89 (m, 4H, aromatic region); LR EIMS (m/z, amu): 279 [M]$^*$ (7%) and 205 [M – CH$_3$SCH = CH$_2$]$^*$ (84%).

(2S)-2-(1,3-Dioxoisooindolin-2-yl)-3-(1H-indol-3-yl)propanoic acid (3e)

Yellow solid, yield (%): 97 (conventional heating), 95 (microwave), Mp: 182 °C (Lit$^2$ 181-183 °C), $R_f = 0.70$ (EtOH); $[\alpha]^{20}$D: $-249.0$ for MW and $-249.9$ for conventional heating.
method (c 1.0, EtOH), (Lit² [x])²⁰_D −249.6, c 1.0, EtOH); log ε (λ_max, nm): 3.2346 (312);
FT-IR (KBr, υ_max/cm⁻¹): 3397 (O−H, N−H), 1711 (C=O); ¹H NMR (400 MHz, CD₃OD, in ppm):
δ_H 2.9 (broad s, 1H, OH), 3.69−3.80 (m, 2H), 5.27 (dd, J = 11.4, J = 4.8 Hz, 1H), 6.95 (m, 1H), 7.03 (m, 1H), 7.10 (d, J = 4.8 Hz, 1H), 7.29 (d, J = 7.8 Hz, 1H), 7.59 (d, J = 7.8 Hz, 1H), 7.78−7.81 (m, 4H), 10.0 (broad s, 1H, NH); ¹³C NMR
(400 MHz, CD₃OD, in ppm): δ_C 24.2, 52.3, 110.2, 111.9, 117.4, 118.5, 121.3, 123.8, 127.1, 130.3, 134.1, 136.2, 167.4, 170.4.

(2S)-2-(1,3-Dioxoisindol-2-yl)-3-phenylpropanoic acid (3f)
White crystals, yield (%): 97 (conventional heating), 95 (microwave), Mp: 184 °C (Lit²) 84−186 °C); R_f = 0.71 (EtOH); [x]²⁹_D −214.0 for MW and −217.3 for conventional heating method (c 1.0, EtOH), (Lit² [x])²²_D −215.7, c 1.52, EtOH); log ε (λ_max, nm): 3.2333 (320); FT-IR (KBr, υ_max/cm⁻¹) 3271 (O−H), 1771, 1700 (C=O); ¹H NMR (400 MHz, CDCl₃, ppm): δ_H 3.60 (d, 2H, J = 9.0 Hz), 5.23 (t, 1H, J = 9.0 Hz), 6.40 (broad s, 1H, −OH), 7.11−7.19 (m, 5H, Ar−H), 7.70 (dd, 2H, J = 5.4 Hz, J = 3.0 Hz), 7.78 (dd, 2H, J = 5.4 Hz, J = 3.0 Hz); ¹³C NMR (100 MHz, CDCl₃, ppm): δ_C 33.8, 52.9, 123.1, 127.2, 128.3, 128.1, 131.7, 133.7, 136.3, 168.0.

Molecular docking
Molecular docking of compounds 3a-f and the reference standard (Sulbactam) with active site 3BLM was achieved using the AutoDock Vina docking algorithm in the program 1-Click Docking (Mcule Inc., Palo Alto, CA, USA). The binding site center was identified as default, with the Cartesian coordinates (X: 2.533, Y: −10.807 and Z: −9.408). The simulation was RUN with a maximum hit of 1000. We selected the docking pose with the most negative docking score corresponding to the highest binding power, and hydrogen bonding was analyzed by Biovia Discovery Studio 2020 (Systemes Dassault, 2016). Details are available in the Supplementary Materials of the online version of this article and from the corresponding author upon request.

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References

1. F. Yassin, A. Seleim, A. Hassan, A. Hadad, N. Fathy and M. Fayad, *Br. J. Pharm. Res.*, 4, 1923 (2014). doi:10.9734/BJPR/2014/6355
2. Q. Zeng, Z. Liu, B. Li and F. Wang, *Amino Acids*, 27, 183 (2004). doi:10.1007/s00726-004-0109-1
3. Y. T. Liu, S. M. Song, D. W. Yin and D. Chen, “Advanced Materials Research,” Vol. 1088, p. 363, Kapellweg, Switzerland, 2015.
4. J. H. Billman and W. F. Harting, *J. Am. Chem. Soc.*, 70, 1473 (1948). doi:10.1021/ja01184a051
5. J. C. Sheehan, D. W. Chapman and R. W. Roth, *J. Am. Chem. Soc.*, 74, 3822 (1952). doi:10.1021/ja01135a031
6. J. Fraga-Dubreuil, G. Çomak, A. W. Taylor and M. Poliakoff, *Green Chem.*, 9, 1067 (2007). doi:10.1039/b704405d
7. A. K. Bose, F. Greer and C. C. Price, *J. Org. Chem.*, 23, 1335 (1958). doi:10.1021/jo01103a025
8. H. M. Al-Hazimi, A. El-Faham, M. Ghazzali and K. Al-Farhan, *Arab. J. Chem.*, 5, 285 (2012). doi:10.1016/j.arabjc.2010.06.020
9. A. C. L. Leite, F. F. Barbosa, M. V. D. O. Cardoso, D. R. M. Moreira, L. C. D. Coelho, E. B. Da Silva, G. B. D. O. Filho, V. M. O. De Souza, V. R. A. Pereira, L. D. C. Reis, P. M. M. Ferreira, C. Pessoa, A. G. Wanderley, F. V. B. Mota and T. G. Da Silva, *Med. Chem. Res.*, 23, 1701 (2014). doi:10.1007/s00044-013-0730-1
10. B. Martin, H. Sekljic and C. Chassaing, *Org. Lett.*, 5, 1851 (2003). doi:10.1021/ol0343818
11. J. R. Casimir, G. Guichard and J.-P. Briand, *J. Org. Chem.*, 67, 3764 (2002). doi:10.1021/jo016347h
12. C. R. McArthur, P.M. Worster and A.U. Okon, *Synth. Commun.*, 13, 393 (1983). doi:10.1080/00397918308066995
13. L. M. Lima, P. Castro, A.L. Machado, C. A. M. Fraga, C. Lugnier, V. L. G. de Moraes and E. J. Barreiro, *Bioorg. Med. Chem.*, 10, 3067 (2002). doi:10.1016/S0968-0896(02)00152-9
14. G. S. Gruzdyev, V. A. Zinchenko and V. A. Kalinin, “The Chemical Protection of Plants” p. 360, G. S. Gruzdyev, Moscow, 1983.
15. U. S. Ramulu Sree, “Chemistry of Insecticides & Fungicides,” p. 48, Scientific Publisher, New Delhi, 2020.
16. O. M. O. Habib, E. B. Moawad, D. S. Badawy and F. A. Mansour, *J. Für Prakt. Chemie.*, 332, 791 (1990). doi:10.1002/prac.199033202531
17. T. C. Barros, S. Brochtszain, V. G. Toscano, P. B. Filho and M. J. Politi, *J. Photochem. Photobiol. A Chem.*, 111, 97 (1997). doi:10.1016/S1010-6030(97)00205-0
18. M. F. Braña, M. Cacho, A. Ramos, M. Teresa Domínguez, J. M. Pozuelo, C. Abradelo, M. Fernanda Rey-Stolle, M. Yuste, C. Carrasco and C. Bailly, *Org. Biomol. Chem.*, 1, 648 (2003). doi:10.1039/b209042b
19. A. Dorlars, C.-W. Schellhammer and J. Schroeder, *Angew. Chemie Int. Ed. English.*, 14, 665 (1975). doi:10.1002/anie.197506651
20. F. Würtchner, Z. Chen, F. J. M. Hoeben, P. Osswald, C.-C. You, P. Jonkheijm, J. V. Herrikhuyzen, A. P. H. J. Schenning, P. P. A. M. van der Schoot, E. W. Meijer, E. H. A. Beckers, S. C. J. Meskers and R. A. J. Janssen, *J. Am. Chem. Soc.*, 126, 10611 (2004). doi:10.1021/ja0475353
21. P. F. Lamie, J. N. Phillopes, A.O. El-Gendy, L. Barova and J. Gruz, *Molecules*, 20, 16620 (2015). doi:10.3390/molecules200916620
22. J. L. Santos, P. R. Yamasaki, C. M. Chin, C. H. Takashi, F. R. Pavan and C. Q. F. Leite, *Bioorg. Med. Chem.*, 17, 3795 (2009). doi:10.1016/j.bmc.2009.04.042
23. T. N. Bansode, J. V Shelke and V. G. Dongre, *Eur. J. Med. Chem.*, 44, 5094 (2009). doi:10.1016/j.ejmech.2009.07.006
24. T. Vidal, A. Petit, A. Loupy and R. N. Gedye, *Tetrahedron*, 56, 5473 (2000). doi:10.1016/S0040-4020(00)00445-2
25. G.W. V Cave, C.L. Raston and J.L. Scott, Chem. Commun., 21, 2159 (2001). doi:10.1039/b106677n
26. C. Imrie, P. Kleyi, V. O. Nyamori, T. I. A. Gerber, D. C. Levendis and J. Look, J. Organomet. Chem., 692, 3443 (2007). doi:10.1016/j.jorganchem.2007.04.011
27. J. O. Metzger, Angew. Chem. Int. Ed. Engl., 37, 2975 (1998). doi:10.1002/(SICI)1521-3773(19981116)37:21<2975::AID-ANIE2975>3.0.CO;2-A
28. C.-J. Li and T. H. Chan, Tetrahedron. 55, 11149 (1999). doi:10.1016/S0040-4020(99)00641-9
29. T.-P. Loh, J. M. Huang, S. H. Goh and J. J. Vittal, Org. Lett., 2,1291 (2000). doi:10.1021/ol000042s
30. R. S. Varma, and V. V. Namboodiri, Pure Appl. Chem., 73, 1309 (2001). doi:10.1351/pac200173081309
31. K. Tanaka and F. Toda, Solvent-Free Organic Synthesis, Chem. Rev., 100, 1025 (2000). doi:10.1021/cr940089p
32. M. B. Deshmukh, S. S. Patil, S. D. Jadhav and P. B. Pawar, Synth. Commun., 42, 1177 (2012). doi:10.1080/00397911.2010.537423
33. R. D. Taylor, P. J. Jewsbury and J. W. Essex, J. Comput. Aided. Mol. Des., 16, 151 (2002). doi:10.1023/A:1020155510718
34. H. Gohlke and G. Klebe, Curr. Opin. Struct. Biol., 11, 231 (2001). doi:10.1016/S0959-440X(00)00195-0
35. G. A. Jacoby, Antimicrob. Agents Chemother., 50, 1123 (2006). doi:10.1128/AAC.50.4.1123-1129.2006
36. K. Bush and G. A. Jacoby, Antimicrob. Agents Chemother., 54, 969 (2010). doi:10.1128/AAC.01009-09
37. S. M. Drawz and R. A. Bonomo, Clin. Microbiol. Rev., 23, 160 (2010). doi:10.1128/CMR.00037-09
38. F. K. Majiduddin, I. C. Materon and T. G. Palzkill, Int. J. Med. Microbiol., 292, 127 (2002). doi:10.1078/1438-4221-00198
39. M. S. Wilke, A. L. Lovering and N. C. J. Strynadka, Curr. Opin. Microbiol., 8, 525 (2005). doi:10.1016/j.mib.2005.08.016
40. J. F. Fisher and S. Mobashery, Curr. Protein Pept. Sci., 10, 401 (2009). doi:10.2174/138920309789351967
41. O. Herzberg, J. Mol. Biol., 217, 701 (1991). doi:10.1016/0022-2836(91)90527-D