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Design and synthesis of potential β-sheet nucleators via Suzuki coupling reaction

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Abstract—Three series of compounds characterized by biphenylic structure were synthesized in order to develop new scaffolds able to induce β-sheet folding in the peptides. Microwave flash heating was used in order to shorten reaction times and to enhance the obtained yields. Simulated annealing molecular dynamics simulations demonstrated that some of the compounds were capable of adopting a 15-membered intramolecularly hydrogen-bonded conformation, which supports an antiparallel β-sheet structure.

1. Introduction

The secondary structure of peptides and proteins is critical for their bioactivities. Several examples are reported in the literature of molecular mimics of specific secondary structures by means of non-peptidic scaffolds.[1–3] In particular, the mechanism of β-sheet folding and the basis for sheet stability are under investigation in several laboratories using a variety of creative approaches.[4] The β-sheet structure is currently an interesting target because of its poor abundance with respect to α-helical secondary structure; our lack of knowledge about β-sheets is due, in part, to difficulties inherent in creating a well defined peptide model system for the detailed study of β-sheet formation in aqueous media.[5]

Although studies on the conformational properties of peptides have enhanced our understanding of β-sheet structure, the approach is limited because of competitive intra- and inter-molecular foldings, which lead to heterogeneous β-sheet structures. Our goal was to develop a new reliable strategy to prepare monomeric β-hairpin structures that can accommodate considerable α-amino acid sequence variations.

However, the conformational properties of known β-turn sequences do not appear to be sufficient to effect β-hairpin folding, given what is currently known about the process. Therefore, several laboratories are interested in designing unnatural amino acid templates in order to initiate β-sheet folding, taking advantage of the template concept first reported by Hirschmann and co-workers in their work on cyclic somatostatin analogues.[6]

Here, we describe the development of a series of compounds characterized by a general structure reported in Figure 1, which have been designed to replace the backbone of a β-turn and reverse the peptide chain direction via a hydrogen-bonded hydrophobic cluster conformation to initiate β-hairpin folding.

The new molecules have a common scaffold, characterized by a biphenyl-based amino acid structure with the carboxyl and amino functions in the *ortho* positions, substituted with electron withdrawing or donating substituents. The introduction of these substituents is important because in this way we can introduce some pharmacologically relevant functional groups in an unconventional position of the peptide.

The synthetic approach for the development of these new compounds has involved the use of microwave flash heating in order to shorten reaction times and to increase the obtained yields.[7] In fact, during recent years, our interests were mainly directed to advances in the syntheses of peptidomimetics and particularly to the application of microwave irradiation in the field of peptide chemistry.[7,8]

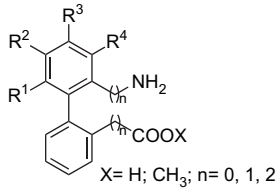

Figure 1. General structure of β-sheet nucleators.

Keywords: β-Sheet mimetics; Microwave irradiation; Suzuki coupling.
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We developed three series of compounds, indicated as **3a–3f**, **4a–4f** and **5a–5f**, respectively, characterized by the carboxylic and the amino groups directly bond to the phenyl ring or by one or two additional methylene groups, respectively.

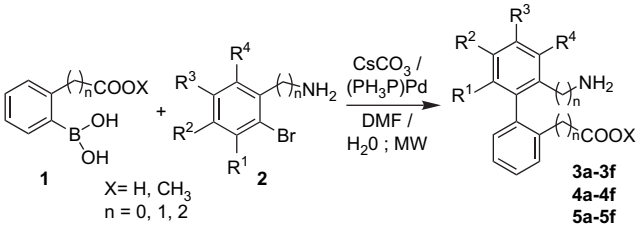
Our efforts in the development of biphenyl structures started from the consideration that similar structures were reported in the literature as able to induce the β-sheet conformation;[6] this was obtained through a H-bond, generating a 13- or 15-membered ring, that favored the β-sheet moiety. Moreover, compound **4a** was already reported as a dipeptide β-turn mimetic and this was consistent with predictions made by molecular modeling calculations.[10]

Molecular modeling calculations have demonstrated that compounds **5a–5f** are capable of adopting a 15-membered intramolecularly hydrogen-bonded conformation, which should support an antiparallel β-sheet structure. These results are in consonance with FTIR and variable temperature ^{1}H NMR spectroscopic studies performed on the diamide derivative of a similar compound, the [3′-(2-aminoethyl)-2-biphenyl]propionic acid,[9] which suggested that this compound is capable of promoting hydrogen-bonded conformations so as to facilitate β-sheet structure formation in CH_2Cl_2.

2. Results and discussion

The syntheses of biphenyl structures with the proposed features were described in the literature as composed of several synthetic steps.[10]

In our approach, instead, we developed the one pot synthesis of biphenyl compounds **3a–3f**, **4a–4f**, and **5a–5f** (Scheme 1) optimizing the conditions of the Suzuki coupling.[11]



Scheme 1. General synthetic procedure of compounds **3a–3f**, **4a–4f**, and **5a–5f** via Suzuki coupling reaction.

Since the examples of Suzuki reactions with aniline halide substrates are quite limited in the literature, several reaction conditions were tried.[12] The best results were obtained in aqueous media using cesium carbonate as base; these conditions were the best also for the development of benzylaminic or phenylethyl aminic derivatives. The presence of electron withdrawing or donating substituents allowed us to investigate their role in the Suzuki coupling. Reactions and conditions are reported in Scheme 1.

The reactions were carried out using a microwave oven (ETHOS 1600, Milestone®) especially designed for organic synthesis. The boronic acid **1** and the appropriate aniline halide or phenyl alkyl halide **2** were dissolved in a mixture of DMF/water in a 5:1 ratio. The reaction mixture, added with cesium carbonate and the palladium catalyst, was then transferred into a sealed tube and irradiated by microwave for a total time of 30 min. Microwave power was set at 300 W and the temperature kept at 150 °C for 30 min.

The main aspect of our procedure was that a short irradiation time of the reaction mixture furnished the final compounds in 30 min, with quite good yields in a single step procedure.

The yields of compounds **3a–3f**, **4a–4f**, and **5a–5f** are summarized in Table 1.

The diamide derivatives **6a**, **7a**, and **8a** (Fig. 2), derived from compounds **3a**, **4a**, and **5a**, respectively, were studied by molecular modeling in an effort to evaluate their potential abilities for β-sheet nucleation as well as to probe their intramolecular hydrogen-bonding preferences.

The conformational space available to these structures is limited by the restricted rotation about the interaromatic single

Table 1. Substituents and obtained yields of compounds **3a–3f**, **4a–4f** and **5a–5f**

Compound	*n*	X	R^1	R^2	R^3	R^4	Yield[a] (%)
3a	0	H	H	H	H	H	34
3b	0	H	H	CF_3	H	H	17
3c	0	H	H	H	NO_2	H	34
3d	0	H	H	CH_3	H	H	29
3e	0	CH_3	H	H	H	CH_3	24
3f	0	CH_3	H	H	CF_3	H	19
4a	1	H	H	H	H	H	37
4b	1	H	H	CF_3	H	H	15
4c	1	H	H	H	NO_2	H	36
4d	1	H	H	CH_3	H	H	30
4e	1	CH_3	H	H	H	CH_3	22
4f	1	CH_3	H	H	CF_3	H	23
5a	2	H	H	H	H	H	39
5b	2	H	H	CF_3	H	H	20
5c	2	H	H	H	NO_2	H	38
5d	2	H	H	CH_3	H	H	33
5e	2	CH_3	H	H	H	CH_3	23
5f	2	CH_3	H	H	CF_3	H	25

[a] All the reactions were performed three times and the reaction time and yields given are the average values.

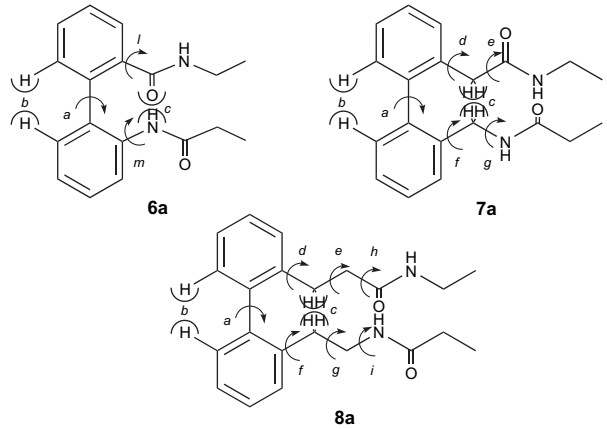


Figure 2. Parameters affecting conformational flexibility and constraint in the diamide derivatives of **6a**, **7a**, and **8a**, designed as β-sheet nucleators. In the biphenyl derivatives, flexibility is provided by free rotation about bonds *a*, *d*, *e*, *f*, *g*, *h*, and *i*, and constraint is imposed by restricted rotation about bonds *l* and *m*, steric clashes *b* and *c* between substituents *ortho* to the interaromatic bond, and possible hydrogen bond between the NH and CO amide groups.

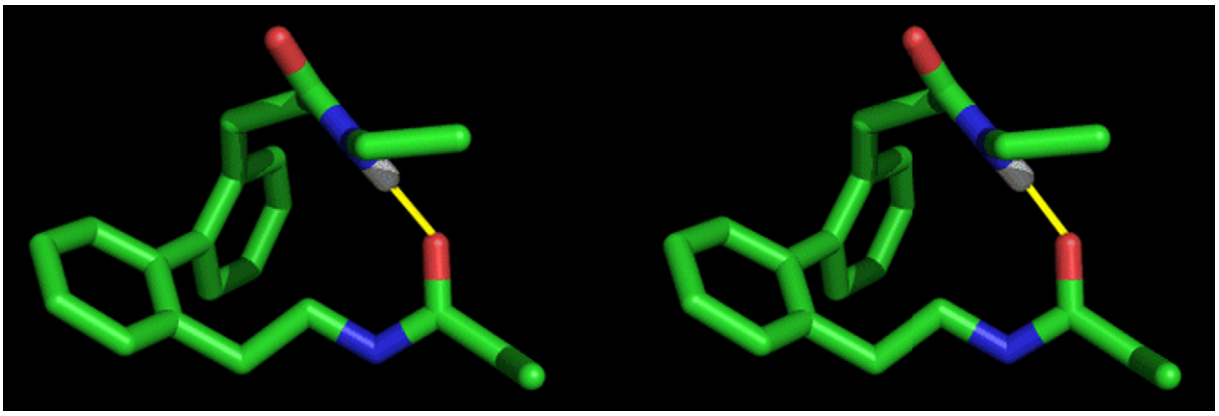


Figure 3. Stereoview of the most highly populated conformation cluster derived from simulated annealing MD calculations for the diamide β-sheet nucleator **8a**. The hydrogen bond, which contributes to form a 15-membered ring between the NH and CO amide groups, is shown as a yellow line. For reasons of clarity only the heavy atoms (C: green, N: blue, O: red) and amide protons (white) are shown.

bond *a* due to a balance of opposing effects. Overlap of the π systems of the rings favors their coplanarity, whereas steric clashes between substituents *ortho* to the interaromatic linkage such as hydrogen atoms and the CO and NH groups (*b* and *c*) disfavor coplanarity. Because the amides are directly attached to the aromatic system in compound **6a**, rotation about bonds *l* and *m* is expected to be constrained to dihedral angles of ±25° between the plane of the amide and that of the aromatic ring. These values for the dihedral angle were observed in a search of relevant small-molecule crystal structures in the Cambridge database, and can be explained by the balance of two opposing forces. Finally, the conformation of the biphenyl system should allow hydrogen bonding to occur between the NH and CO amide groups. Such a hydrogen bond corresponds to that between residues *i* and *i*+3 of a β-hairpin, and its presence would provide further conformational constraint on the system.

By simulated annealing molecular dynamics (MD) simulation over 5000 ps, the stability of the β-hairpin pattern was investigated in the gas phase for compounds under discussion.

Whereas diamides **6a** and **7a** do not fold into a stable hairpin structure over the trajectory of the MD simulation, the β-hairpin pattern remains stable for diamide **8a** (most populated cluster with 88% occupancy along the trajectory).

As depicted in Figure 3, the diamide derivative **8a** is capable of adopting a 15-membered intramolecularly hydrogen-bonded conformation, which should support an antiparallel β-sheet structure. These results are consistent with FTIR and variable temperature ^{1}H NMR spectroscopic studies performed on the diamide derivative of a similar compound, the [3′-(2-aminoethyl)-2-biphenyl]propionic acid,[9] which suggested that this compound is capable of promoting hydrogen-bonded conformations so to facilitate β-sheet structure formation in CH_2Cl_2.

3. Experimental section

3.1. Microwave equipment and conditions

The synthetic steps performed by microwave irradiation were carried out using a microwave oven (ETHOS 1600, Milestone®) especially designed for organic synthesis. Microwave reactions were performed in sealed tubes and a microwave program that was composed by appropriate ramping and holding steps was selected. The temperature of the stirred reaction mixture was monitored directly by a microwave-transparent fluoroptic probe inserted into the reaction mixture; irradiation time and power were monitored with the 'easyWAVE' software package.

3.2. Chemistry

All reactions were followed by thin layer chromatography carried out on Merck silica gel 60 F_{254} plates with fluorescent indicator and the plates were visualized with UV light (254 nm). Preparative chromatographic purifications were performed using silica gel column (Kieselegel 60). Melting points were determined using a Kofler hot-stage apparatus. Elemental analyses were carried out on a Carlo Erba model 1106. The structures were verified spectroscopically by ^{1}H NMR, ^{13}C NMR, IR, and ESI-MS; in ^{13}C NMR of fluorinated compounds, carbon–fluorine decoupling was applied. Spectra were recorded on a Varian Mercury Plus 400 MHZ instrument. Chemical shifts are given as δ; the solvent was DMSO-d_6. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), br s (broad singlet), t (triplet). Mass spectra of the final products were performed on LCQ Thermoquest-Ion trapmass spectrometry. IR spectra were recorded on Thermo Nicolet 5700 FT-IR spectrometer.

3.3. General procedure for the microwave synthesis of compounds 3a–3f, 4a–4f, and 5a–5f

The boronic acid **1** (2.22 mmol) and the appropriate aniline halide or phenyl alkyl halide **2** (1.48 mmol) were dissolved in a mixture of DMF/water (5:1 v/v). Cesium carbonate (5.92 mmol) and tetrakis (triphenylphosphine) palladium catalyst (0.044 mmol) were added to the reaction mixture that was stirred for 5 min at room temperature. The reaction was then transferred into a sealed vial specifically designed for reactions with high pressure. The mixture was irradiated at 150 °C by microwave for 30 min at 300 W. The solvent was removed under reduced pressure and the residue obtained was taken up in dichloromethane and extracted (three

times) with $NaHCO_3$ 5% and brine. The organic phase was dried over Na_2SO_4, filtered, and dried in vacuo. The mixture was purified over an opened silica gel column affording final compounds **3a–3f**, **4a–4f**, and **5a–5f**.

3.3.1. 2-(2-Aminophenyl)benzoic acid (3a). Yield 107 mg, 34%, white powder, mp 133–135 °C; R_f 0.52 (*n*-hexane/ethyl acetate 6:4); ν_{max} (KBr) 3425, 3321, 2956, 2852, 1686, 1619, 1258, 1084, 795 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.24 (1H, t, *J* 8.0 Hz), 7.34 (1H, d, *J* 8.0 Hz), 7.47 (1H, t, *J* 8.0 Hz), 7.63 (1H, t, *J* 7.8 Hz), 7.84 (1H, t, *J* 7.8 Hz), 8.30 (1H, d, *J* 8.0 Hz), 8.37 (1H, d, *J* 7.8 Hz), 8.49 (1H, d, *J* 7.8 Hz), 9.62 (br s, 1H, OH), 11.67 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 116.8, 118.2, 122.9, 123.3, 123.9, 126.3, 128.1, 128.6, 130.3, 133.5, 134.9, 137.2, 161.5; MS: 214 (MH^+), 236 (M−Na), 252 (M−K). Anal. Calcd for $C_{13}H_{11}NO_2$: C, 73.23; H, 5.20; N, 6.57. Found: C, 73.19; H, 5.23; N, 6.52.

3.3.2. 2-(2-Amino-5-(trifluoromethyl)phenyl)benzoic acid (3b). Yield 71 mg, 17%, white powder, mp 168–170 °C; R_f 0.73 (ethyl acetate/petroleum ether 5:5); ν_{max} (KBr) 3423, 3320, 2932, 2850, 1678, 1410, 1239, 1118, 794 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.34 (1H, d, *J* 7.9 Hz), 7.47 (s, 1H), 7.63 (1H, t, *J* 7.8 Hz), 7.84 (1H, t, *J* 7.8 Hz), 8.30 (1H, d, *J* 7.9 Hz), 8.37 (1H, d, *J* 7.8 Hz), 8.49 (1H, d, *J* 7.8 Hz), 9.58 (br s, 1H, OH), 11.64 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 116.8, 118.2, 122.9, 123.3, 123.9, 124.5, 126.3, 128.1, 128.6, 130.3, 133.5, 134.9, 137.2, 161.5; MS: 282 (MH^+), 304 (M−Na), 320 (M−K). Anal. Calcd for $C_{14}H_{10}F_3NO_2$: C, 59.79; H, 3.58; N, 4.98. Found: C, 60.01; H, 3.60; N, 5.02.

3.3.3. 2-(2-Amino-4-(nitro)phenyl)benzoic acid (3c). Yield 130 mg, 34%, white powder, mp 159–161 °C; R_f 0.82 (petroleum ether/ethyl acetate 7:3); ν_{max} (KBr) 3424, 3320, 2935, 2852, 1690, 1618, 1507, 1329, 1258, 1088, 791 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.47 (s, 1H), 7.56 (1H, d, *J* 8.2 Hz), 7.85 (1H, t, *J* 8.2 Hz), 8.07 (1H, t, *J* 8.2 Hz), 8.37 (1H, d, *J* 8.2 Hz), 8.49 (1H, d, *J* 7.9 Hz), 8.50 (1H, d, *J* 7.9 Hz), 9.32 (br s, 1H, OH), 11.72 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 117.1, 118.5, 123.1, 123.6, 124.1, 126.5, 128.4, 128.9, 130.5, 133.7, 135.0, 137.4, 161.7; MS: 259 (MH^+), 281 (M−Na), 297 (M−K). Anal. Calcd for $C_{13}H_{10}N_2O_4$: C, 60.47; H, 3.90; N, 10.85. Found: C, 60.53; H, 3.92; N, 10.95.

3.3.4. 2-(2-Amino-5-(methyl)phenyl)benzoic acid (3d). Yield 97 mg, 29%, white powder, mp 108–110 °C; R_f 0.69 (n-hexane/ethyl acetate 6:4); ν_{max} (KBr) 3422, 3318, 2875, 1686, 1417, 1274, 793 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.35 (s, 3H), 7.35 (1H, d, *J* 8.0 Hz), 7.48 (s, 1H), 7.64 (1H, t, *J* 7.8 Hz), 7.85 (1H, t, *J* 7.8 Hz), 8.31 (1H, d, *J* 8.0 Hz), 8.38 (1H, d, *J* 7.8 Hz), 8.50 (1H, d, *J* 7.8 Hz), 9.43 (br s, 1H, OH), 11.42 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 24.6, 116.8, 118.3, 122.9, 123.4, 123.8, 126.1, 128.2, 128.8, 130.4, 133.3, 134.7, 137.4, 161.5; MS: 228 (MH^+), 250 (M−Na), 266 (M−K). Anal. Calcd for $C_{14}H_{13}NO_2$: C, 73.99; H, 5.77; N, 6.16. Found: C, 74.05; H, 5.82; N, 6.23.

3.3.5. 2-(2-Amino-3-(methyl)phenyl)methylbenzoate (3e). Yield 86 mg, 24%, white powder, mp 191–193 °C; R_f 0.69 (ethyl acetate/petroleum ether 5:5); ν_{max} (KBr) 3437, 3328, 2936, 1712, 1436, 1316, 1279, 1071, 758 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.78 (s, 3H), 3.31 (s, 3H), 7.24 (1H, t, *J* 7.7 Hz), 7.47 (1H, t, *J* 7.7 Hz), 7.63 (1H, t, *J* 7.9 Hz), 7.84 (1H, d, *J* 7.7 Hz), 8.30 (1H, d, *J* 7.9 Hz), 8.37 (1H, d, *J* 7.9 Hz), 8.49 (1H, d, *J* 7.7 Hz), 11.67 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 15.5, 39.0, 116.8, 118.2, 122.9, 123.3, 123.9, 126.3, 128.1, 128.6, 130.3, 133.5, 134.9, 137.2, 161.5; MS: 242 (MH^+), 264 (M−Na), 280 (M−K). Anal. Calcd for $C_{15}H_{15}NO_2$: C, 74.67; H, 6.27; N, 5.81. Found: C, 74.52; H, 7.01; N, 5.90.

3.3.6. 2-(2-Amino-4-(trifluoromethyl)phenyl)methylbenzoate (3f). Yield 83 mg, 19%, white powder, mp 121–123 °C; R_f 0.85 (petroleum ether/ethyl acetate 7:3); ν_{max} (KBr) 3437, 3359, 2948, 2859, 1718, 1417, 1253, 1066, 780 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 3.30 (s, 3H), 7.28 (s, 1H), 7.32 (1H, d, *J* 8.0 Hz), 7.65 (1H, t, *J* 7.7 Hz), 7.81 (1H, t, *J* 7.7 Hz), 8.28 (1H, d, *J* 8.0 Hz), 8.34 (1H, d, *J* 7.7 Hz), 8.47 (1H, d, *J* 7.7 Hz), 11.67 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 39.0, 116.8, 118.2, 122.9, 123.3, 123.9, 124.2, 126.3, 128.1, 128.6, 130.3, 133.5, 134.9, 137.2, 161.5; MS: 296 (MH^+), 318 (M−Na), 334 (M−K). Anal. Calcd for $C_{15}H_{12}F_3NO_2$: C, 61.02; H, 4.10; N, 4.74. Found: C, 61.03; H, 4.18; N, 4.68.

3.3.7. 2-(2-(2-Aminomethyl)phenyl)acetic acid (4a). Yield 132 mg, 37%, white powder, mp 139–141 °C; R_f 0.86 (diethyl ether/*n*-hexane 8:2); ν_{max} (KBr) 3027, 2933, 2844, 1719, 1641, 1432, 1231, 1150, 866, 723 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 3.41 (s, 2H), 3.90 (s, 2H), 7.22 (1H, t, *J* 7.8 Hz), 7.32 (1H, d, *J* 7.8 Hz), 7.43 (1H, t, *J* 7.8 Hz), 7.61 (1H, t, *J* 7.5 Hz), 7.82 (1H, t, *J* 7.5 Hz), 8.28 (1H, d, *J* 7.8 Hz), 8.35 (1H, d, *J* 7.5 Hz), 8.47 (1H, d, *J* 7.5 Hz), 9.66 (br s, OH), 11.79 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 41.6, 45.9, 117.1, 118.4, 123.2, 123.7, 124.3, 126.8, 128.4, 129.0, 130.5, 133.8, 135.2, 137.6, 161.8; MS: 241 (MH^+), 264 (M−Na), 280 (M−K). Anal. Calcd for $C_{15}H_{15}NO_2$: C, 74.67; H, 6.27; N, 5.81. Found: C, 74.28; H, 6.82; N, 5.80.

3.3.8. 2-(2-(2-Aminomethyl)-5(trifluoromethyl)phenyl)-acetic acid (4b). Yield 69 mg, 15%, white powder, mp 156–158 °C; R_f 0.85 (ethyl acetate/petroleum ether 5:5); ν_{max} (KBr) 3026, 2963, 1708, 1465, 1241, 1136, 1029, 754 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 3.41 (s, 2H), 3.90 (s, 2H), 7.34 (1H, d, *J* 7.9 Hz), 7.47 (s, 1H), 7.63 (1H, t, *J* 7.8 Hz), 7.84 (1H, t, *J* 7.8 Hz), 8.30 (1H, d, *J* 7.9 Hz), 8.37 (1H, d, *J* 7.8 Hz), 8.49 (1H, d, *J* 7.8 Hz), 9.58 (br s, 1H, OH), 11.64 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 41.6, 45.9, 116.8, 118.2, 122.9, 123.3, 123.9, 124.5, 126.3, 128.1, 128.6, 130.3, 133.5, 134.9, 137.2, 161.5; MS: 310 (MH^+), 332 (M−Na), 348 (M−K). Anal. Calcd for $C_{16}H_{14}F_3NO_2$: C, 62.13; H, 4.56; N, 4.53. Found: C, 62.25; H, 4.83;N, 4.62.

3.3.9. 2-(2-(2-Aminomethyl)-4(nitro)phenyl)acetic acid (4c). Yield 152 mg, 36%, white powder, mp 181–183 °C; R_f 0.88 (petroleum ether/ethyl acetate 7:3); ν_{max} (KBr) 3026, 2931, 1713, 1526, 1344, 1315, 1283, 1056, 785 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 3.41 (s, 2H), 3.90 (s, 2H), 7.47 (s, 1H), 7.56 (1H, d, *J* 8.2 Hz), 7.85 (1H, t, *J* 8.2 Hz), 8.07 (1H, t, *J* 7.9 Hz), 8.37 (1H, d, *J* 8.2 Hz),

8.49 (1H, d, *J* 7.9 Hz), 8.50 (1H, d, *J* 7.9 Hz), 9.32 (br s, 1H, OH), 11.72 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 41.6, 45.9, 117.1, 118.5, 123.1, 123.6, 124.1, 126.5, 128.4, 128.9, 130.5, 133.7, 135.0, 137.4, 161.7; MS: 287 (MH^+), 309 (M−Na), 325 (M−K). Anal. Calcd for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.79. Found: C, 63.03; H, 4.90; N, 9.56.

3.3.10. 2-(2-(2-Aminomethyl)-5(methyl)phenyl)acetic acid (4d). Yield 113 mg, 30%, white powder, mp 123–125 °C; R_f 0.76 (n-hexane/ethyl acetate 6:4); ν_{max} (KBr) 3034, 2942, 1712, 1636, 1478, 1137, 816 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.35 (s, 3H), 3.41 (s, 2H), 3.90 (s, 2H), 7.35 (1H, d, *J* 8.0 Hz), 7.48 (s, 1H), 7.64 (1H, t, *J* 7.8 Hz), 7.85 (1H, t, *J* 7.8 Hz), 8.31 (1H, d, *J* 8.0 Hz), 8.38 (1H, d, *J* 7.8 Hz), 8.50 (1H, d, *J* 7.8 Hz), 9.43 (br s, 1H, OH), 11.42 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 24.6, 41.6, 45.9, 116.8, 118.3, 122.9, 123.4, 123.8, 126.1, 128.2, 128.8, 130.4, 133.3, 134.7, 137.4, 161.5; MS: 256 (MH^+), 278 (M−Na), 294 (M−K). Anal. Calcd for $C_{16}H_{17}NO_2$: C, 75.27; H, 6.71; N, 5.49. Found: C, 75.13; H, 6.73; N, 5.54.

3.3.11. 2-(2-(2-Aminomethyl)-3(methyl)phenyl)acetate (4e). Yield 88 mg, 22%, white powder, mp 138–140 °C; R_f 0.76 (ethyl acetate/petroleum ether 5:5); ν_{max} (KBr) 3021, 2927, 1737, 1613, 1310, 1221, 1030, 745 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.78 (s, 3H), 3.31 (s, 3H), 3.41 (s, 2H), 3.90 (s, 2H), 7.24 (1H, t, *J* 7.7 Hz), 7.47 (1H, t, *J* 7.7 Hz), 7.63 (1H, t, *J* 7.9 Hz), 7.84 (1H, d, *J* 7.7 Hz), 8.30 (1H, d, *J* 7.9 Hz), 8.37 (1H, d, *J* 7.9 Hz), 8.49 (1H, d, *J* 7.7 Hz), 11.67 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 15.5, 39.0, 41.6, 45.9, 116.8, 118.2, 122.9, 123.3, 123.9, 126.3, 128.1, 128.6, 130.3, 133.5, 134.9, 137.2, 161.5; MS: 270 (MH^+), 292 (M−Na), 308 (M−K). Anal. Calcd for $C_{17}H_{19}NO_2$: C, 75.81; H, 7.11; N, 5.20. Found: C, 75.64; H, 7.19; N, 5.36.

3.3.12. 2-(2-(2-Aminomethyl)-4(trifluoromethyl)phenyl)-acetate (4f). Yield 110 mg, 23%, white powder, mp 166–168 °C; R_f 0.91 (petroleum ether/ethyl acetate 7:3); ν_{max} (KBr) 3039, 2931, 1743, 1618, 1458, 1283, 1119, 776 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.30 (s, 3H), 3.41 (s, 2H), 3.90 (s, 2H), 7.28 (s, 1H), 7.32 (1H, d, *J* 8.0 Hz), 7.65 (1H, t, *J* 7.7 Hz), 7.81 (1H, t, *J* 7.7 Hz), 8.28 (1H, d, *J* 8.0 Hz), 8.34 (1H, d, *J* 7.7 Hz), 8.47 (1H, d, *J* 7.7 Hz), 11.67 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 39.0, 41.6, 45.9, 116.8, 118.2, 122.9, 123.3, 123.9, 124.2, 126.3, 128.1, 128.6, 130.3, 133.5, 134.9, 137.2, 161.5; MS: 324 (MH^+), 346 (M−Na), 362 (M−K). Anal. Calcd for $C_{17}H_{16}F_3NO_2$: C, 63.15; H, 4.99; N, 4.33. Found: C, 63.32; H, 4.73; N, 4.52.

3.3.13. 3-2-(2-(2-Aminomethyl)phenyl)propionic acid (5a). Yield 155 mg, 39%, white powder, mp 138–140 °C; R_f 0.92 (diethyl ether/n-hexane 8:2); ν_{max} (KBr) 3026, 2963, 2850, 1706, 1686, 1584, 1475, 1271, 796, 768 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.64 (2H, t, *J* 8.6 Hz), 2.82 (2H, t, *J* 8.3 Hz), 3.41 (2H, t, *J* 8.6 Hz), 3.90 (2H, t, *J* 8.3 Hz), 7.22 (1H, t, *J* 7.8 Hz), 7.32 (1H, d, *J* 7.8 Hz), 7.43 (1H, t, *J* 7.8 Hz), 7.61 (1H, t, *J* 7.5 Hz), 7.82 (1H, t, *J* 7.5 Hz), 8.28 (1H, d, *J* 7.8 Hz), 8.35 (1H, d, *J* 7.5 Hz), 8.47 (1H, d, *J* 7.5 Hz), 9.62 (br s, OH), 11.71 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 26.9, 31.8, 36.7, 36.9, 117.1, 118.4, 123.2, 123.7, 124.3, 126.8, 128.4, 129.0, 130.5, 133.8, 135.2, 137.6, 161.8; MS: 270 (MH^+), 292 (M−Na), 308 (M−K). Anal. Calcd for $C_{17}H_{19}NO_2$: C, 75.81; H, 7.12; N, 5.20. Found: C, 75.22; H, 7.32; N, 5.44.

3.3.14. 3-2-(2-(2-Aminomethyl)-5(trifluoromethyl)phenyl)propionic acid (5b). Yield 100 mg, 20%, white powder, mp 178–180 °C; R_f 0.87 (ethyl acetate/petroleum ether 5:5); ν_{max} (KBr) 3022, 2948, 1710, 1612, 1286, 1183, 802 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.64 (2H, t, *J* 8.6 Hz), 2.82 (2H, t, *J* 8.3 Hz), 3.41 (2H, t, *J* 8.6 Hz), 3.90 (2H, t, *J* 8.3 Hz), 7.34 (1H, d, *J* 7.9 Hz), 7.47 (s, 1H), 7.63 (1H, t, *J* 7.8 Hz), 7.84 (1H, t, *J* 7.8 Hz), 8.30 (1H, d, *J* 7.9 Hz), 8.37 (1H, d, *J* 7.8 Hz), 8.49 (1H, d, *J* 7.8 Hz), 9.58 (br s, 1H, OH), 11.64 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 26.9, 31.8, 36.7, 36.9, 116.8, 118.2, 122.9, 123.3, 123.9, 124.5, 126.3, 128.1, 128.6, 130.3, 133.5, 134.9, 137.2, 161.5; MS: 338 (MH^+), 360 (M−Na), 376 (M−K). Anal. Calcd for $C_{18}H_{18}F_3NO_2$: C, 64.09; H, 5.38; N, 4.15. Found: C, 64.16; H, 5.22;N, 4.33.

3.3.15. 3-2-(2-(2-Aminomethyl)-4(nitro)phenyl)propionic acid (5c). Yield 177 mg, 38%, white powder, mp 142–144 °C; R_f 0.92 (petroleum ether/ethyl acetate 7:3); ν_{max} (KBr) 3031, 2961, 1703, 1528, 1346, 1258, 1083, 769 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.64 (2H, t, *J* 8.6 Hz), 2.82 (2H, t, *J* 8.3 Hz), 3.41 (2H, t, *J* 8.6 Hz), 3.90 (2H, t, *J* 8.3 Hz), 7.47 (s, 1H), 7.56 (1H, d, *J* 8.2 Hz), 7.85 (1H, t, *J* 8.2 Hz), 8.07 (1H, t, *J* 7.9 Hz), 8.37 (1H, d, *J* 8.2 Hz), 8.49 (1H, d, *J* 7.9 Hz), 8.50 (1H, d, *J* 7.9 Hz), 9.32 (br s, 1H, OH), 11.72 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 26.9, 31.8, 36.7, 36.9, 117.1, 118.5, 123.1, 123.6, 124.1, 126.5, 128.4, 128.9, 130.5, 133.7, 135.0, 137.4, 161.7; MS: 315 (MH^+), 337 (M−Na), 353 (M−K). Anal. Calcd for $C_{17}H_{18}N_2O_4$: C, 64.96; H, 5.77; N, 8.91. Found: C, 64.75; H, 5.83; N, 9.02.

3.3.16. 3-2-(2-(2-Aminomethyl)-5(methyl)phenyl)propionic acid (5d). Yield 138 mg, 33%, white powder, mp 158–160 °C; R_f 0.83 (n-hexane/ethyl acetate 6:4); ν_{max} (KBr) 3024, 2956, 1709, 1611, 1489, 1168, 879, 793 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.35 (s, 3H), 2.64 (2H, t, *J* 8.6 Hz), 2.82 (2H, t, *J* 8.3 Hz), 3.41 (2H, t, *J* 8.6 Hz), 3.90 (2H, t, *J* 8.3 Hz), 7.35 (1H, d, *J* 8.0 Hz), 7.48 (s, 1H), 7.64 (1H, t, *J* 7.8 Hz), 7.85 (1H, t, *J* 7.8 Hz), 8.31 (1H, d, *J* 8.0 Hz), 8.38 (1H, d, *J* 7.8 Hz), 8.50 (1H, d, *J* 7.8 Hz), 9.43 (br s, 1H, OH), 11.42 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 24.6, 26.9, 31.8, 36.7, 36.9, 116.8, 118.3, 122.9, 123.4, 123.8, 126.1, 128.2, 128.8, 130.4, 133.3, 134.7, 137.4, 161.5; MS: 284 (MH^+), 306 (M−Na), 322 (M−K). Anal. Calcd for $C_{18}H_{21}NO_2$: C, 76.29; H, 7.47; N, 4.94. Found: C, 76.33; H, 7.41; N, 4.86.

3.3.17. 3-2-(2-(2-Aminomethyl)-3(methyl)phenyl)propanoate (5e). Yield 101 mg, 23%, white powder, mp 188–190 °C; R_f 0.84 (ethyl acetate/petroleum ether 5:5); ν_{max} (KBr) 3039, 2963, 1743, 1640, 1312, 1239, 1088, 796 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.64 (2H, t, *J* 8.6 Hz), 2.78 (s, 3H), 2.82 (2H, t, *J* 8.3 Hz)3.31 (s, 3H), 3.41 (2H, t, *J* 8.6 Hz), 3.90 (2H, t, *J* 8.3 Hz), 7.24 (1H, t, *J* 7.7 Hz), 7.47 (1H, t, *J* 7.7 Hz), 7.63 (1H, t, *J* 7.9 Hz), 7.84 (1H, d, *J* 7.7 Hz), 8.30 (1H, d, *J* 7.9 Hz), 8.37 (1H, d, *J* 7.9 Hz), 8.49 (1H, d, *J* 7.7 Hz), 11.67 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 15.5, 26.9, 31.8, 36.7, 36.9, 116.8, 118.2, 122.9, 123.3, 123.9, 126.3, 128.1, 128.6, 130.3,

133.5, 134.9, 137.2, 161.5; MS: 298 (MH^+), 320 (M−Na), 336 (M−K). Anal. Calcd for $C_{19}H_{23}NO_2$: C, 76.73; H, 7.80; N, 4.71. Found: C, 76.65; H, 7.92; N, 4.76.

3.3.18. 3-2-(2-(2-Aminomethyl)-4(trifluoromethyl)phenyl)propanoate (5f). Yield 130 mg, 25%, white powder, mp 113–115 °C; R_f 0.66 (petroleum ether/ethyl acetate 9:1); ν_{max} (KBr) 3036, 2970, 1740, 1602, 1438, 1259, 1132, 738 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.64 (2H, t, J 8.6 Hz), 2.82 (2H, t, J 8.3 Hz), 3.30 (s, 3H), 3.41 (2H, t, J 8.6 Hz), 3.90 (2H, t, J 8.3 Hz), 7.28 (s, 1H), 7.32 (1H, d, J 8.0 Hz), 7.65 (1H, t, J 7.7 Hz), 7.81 (1H, t, J 7.7 Hz), 8.28 (1H, d, J 8.0 Hz), 8.34 (1H, d, J 7.7 Hz), 8.47 (1H, d, J 7.7 Hz), 11.67 (br s, NH); ^{13}C NMR (DMSO-d_6): δ 26.9, 31.8, 36.7, 36.9, 39.0, 116.8, 118.2, 122.9, 123.3, 123.9, 124.2, 126.3, 128.1, 128.6, 130.3, 133.5, 134.9, 137.2, 161.5; MS: 352 (MH^+), 374 (M−Na), 390 (M−K). Anal. Calcd for $C_{19}H_{20}F_3NO_2$: C, 64.95; H, 5.74; N, 3.99. Found: C, 64.90; H, 5.80; N, 3.91.

3.4. Molecular modeling studies

Molecular modeling and graphics manipulations were performed using the SYBYL software packages[13] running on a Silicon Graphics Tezro workstation equipped with four 700 MHz R16000 processors. Model building of diamides **6a**, **7a**, and **8a** was accomplished with the TRIPOS force field[14] available within SYBYL. Energy minimizations and simulated annealing molecular dynamics (MD) simulations were performed with the AMBER 8.0 program,[15] using the parm99 force field.[16]

Molecular models of enantiomers of diamides **6a**, **7a**, and **8a** were constructed using standard bond lengths and bond angles of the SYBYL fragment library. Geometry optimizations were realized with the SANDER module of AMBER and setting a rms gradient of the forces acting on each atom of 0.01 kcal/mol as the convergence criterion. AM1-BCC charges were assigned to ligands by using the ANTECHAMBER module[17] in AMBER.

The simulated annealing lasted 5000 ps and consisted of three steps: the molecules were fast heated at 1200 K and then were cooled to 0 K over the first 1000 ps, annealed at this temperature for the next 1000 ps and slowly cooled over the next 3000 ps. After cooling, the final conformation obtained was energy-refined using a conjugated gradient algorithm with a final gradient of 0.001 kcal/mol as the convergence criteria. The structures that lie within 5 kcal/mol of the lowest energy conformation of each compound were analyzed further. The obtained minima were compared and clustered into families according to their heavy atom root mean square values Intramolecular amide–amide hydrogen bonds were monitored, the existence of a hydrogen bond being defined by a maximum distance of 0.25 nm between the hydrogen and the acceptor atoms and a minimum angle of 135° between the donor, hydrogen, and acceptor.

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