Synthesis, biological evaluation and docking study of 3-aroyl-1-(4-sulfamoylphenyl)thiourea derivatives as 15-lipoxygenase inhibitors

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A B S T R A C T

A series of 3-aroyl-1-(4-sulfamoylphenyl)thiourea derivatives containing sulfonamide moiety were designed and synthesized as 15-lipoxygenase (15-LOX) inhibitors. Most synthesized compounds showed potent activity against soybean 15-LOX with IC50 values less than 25 μM. The most potent compound 4c (3-methylbenzoyl derivative) with IC50 value of 1.8 μM was 10-fold more potent than quercetin. Interestingly, compound 4c also showed the highest antioxidant activity, as determined by ferric reducing antioxidant power (FRAP) assay. Its capacity for reducing ferric ion was more than ascorbic acid. The viability assay of the selected compound 4c against oxidative stress-induced cell death in differentiated PC12 cells revealed that compound 4c significantly protected neurons against cell death in low concentrations.

1. Introduction

Mammalian lipoxygenases (LOXs) belong to a family of non-heme iron-containing dioxygenases, which catalyze the hydroperoxidation of polyunsaturated fatty acids such as arachidonic and linoleic acids to related hydroperoxides [1]. A heterogeneous family of LOXs was found as 5-LOX, 12-LOX and 15-LOX isoforms which oxidize different position of the key substrate, arachidonic acid [2]. The LOX isoforms have been shown to be involved in the physiopathology and progression of several diseases in human thus would be emerged as an attractive target for therapeutic intervention [3]. Among them, 15-LOX has been implicated in cardiovascular complications (such as atherosclerosis), progression of certain cancers and chronic obstructive pulmonary disease (COPD) [4,5]. Moreover, oxidation of arachidonic and linoleic acids by 15-LOX resulted in metabolites which have been shown to be pro-inflammatory [6] and pro-thrombotic [7]. Accordingly, the finding of new 15-LOX inhibitors has been interesting field in medicinal chemistry and drug discovery.

Thiourea and sulfonamide derivatives have continuously absorbed attention of the medicinal chemists in view of their intense range of biological activities [8–10]. Thiourea derivatives have been employed as anti-inflammatory and antimicrobial [11], antimalarial [12], antitumoral [13], pesticidal [14], and anticancer agents [15]. Also sulfonamides comprise a significant class of drugs with diverse biological properties such as antimicrobial [16,17], anticancer [18,19], anti-inflammatory [20], and antiviral activities.
as well as HIV protease inhibitors [21]. Previously, several sulfonamide-based compounds have been reported as 15-LOX inhibitors [22–24]. Considering the above-mentioned findings about importance of thiourea derivatives especially as anti-inflammatory and lipoxigenase inhibitory compounds, we designed novel phenylthiourea derivatives containing sulfonamide moiety as 15-LOX inhibitors. Since there is a polar cavity in the active site of lipoxigenase enzyme, thus the hydrophilic sulfonamide group as a proton acceptor or donor was connected to the phenylthiourea scaffold to combine their beneficial effects.

In this paper, we described synthesis, biological evaluation and docking study of 3-aryloyl-1-(4-sulfamoylphenyl)thiourea derivatives 4a–o as 15-LOX inhibitors.

2. Results and discussion

2.1. Chemistry

The synthetic route to target compounds namely 3-aryloyl-1-(4-sulfamoylphenyl)thiourea derivatives 4a–o is illustrated in Scheme 1. Firstly, aryl isothiocyanate derivatives 2 were conveniently synthesized using different aryl chlorides 1 and ammonium thiocyanate. Then, compounds 2a–o were reacted with 4-sulfamoylaniline 3 to afford final compounds 4a–o. Compounds 4a–d, 4f, 4g, 4j, 4k, and 4n have been previously reported by Saeed et al. [25]. All final compounds 4a–o were characterized by 1H NMR, 13C NMR, MS spectral data as well as elemental analyses.

2.2. Biological activity

2.2.1. In vitro 15-lipoxygenase inhibitory activity

The inhibitory activity of compounds 4a–o against soybean 15-LOX was expressed as IC50 values in Table 1. Most of benzoylthiourea derivatives 4a–l showed potent activity (IC50 values <25 μM). The 3-methylbenzoyl derivative 4c with IC50 value of 1.8 μM was the most potent compound. Its activity was 10-fold more than that of standard anti-LOX agent quercetin. Moreover, compounds 4a, 4b, 4g, 4h, and 4j were more active than quercetin. Also, compound 4i with IC50 value of 18.7 μM was as potent as quercetin.

As seen from data, the 3-methyl substituent on benzoyl moiety could significantly increase the inhibitory activity against 15-LOX, but other substituent on different position of benzoyl group could not improve the activity. Indeed, 2-methyl, 3-fluoro, 3-chloro derivatives (compounds 4b, 4g, and 4j, respectively) were as potent as unsubstituted analog 4a. By comparing the activity of compounds containing substituent at ortho-, meta-, or para-position of benzoyl group, it revealed that the substitution at meta-position is well tolerated. The replacement of phenyl with 2-naphthyl, 2-furyl and 2-thienyl drastically diminished the activity (compounds 4m–o vs. 4a). In the case of 2-substituted compounds (4b and 4i), the methyl substituent was better than chloro group. In addition, among the 3-substituted compounds (4c, 4g, 4j), the methyl analog 4c had superior activity. Thus at both positions (ortho and meta), the better results were obtained by methyl substituent.

2.2.2. Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) assay was used to determine the total antioxidant potential of the target compounds 4a–o. In this method, the ferric tripyridyl triazine complex is reduced by the test compound to ferrous form which has a deep blue color [26]. The change of absorbance is measured at 585 nm. Results are expressed in mmole ferrous/g dry mass of compounds according to the plotted standard curve of ferrous sulfate (Table 1). Ascorbic acid was used as references drug. Compound 4c showed the highest antioxidant activity, as determined by FRAP assay. Its capacity for reducing ferric ion was more than ascorbic acid. Nevertheless, compounds 4k and 4l also showed high potency for ferric reduction. The comparison of obtained FRAP values for the unsubstituted compound 4a and substituted analogs 4b–l, revealed that the antioxidant potential was occasionally increased by substitution on benzoyl moiety.

2.2.3. Protection against H2O2-induced cell death in PC12 neurons

The neuroprotective activity of the selected compound 4c against oxidative stress-induced cell death in differentiated PC12 cells was evaluated. The differentiated PC12 cells were incubated with different concentrations (1, 5 and 10 μM) of the compound for 3 h prior to treatment with H2O2. Moreover, quercetin was used as reference drug at the concentration of 5 μM. The cell viability was measured after 24 h by using the MTT assay. The data are shown in Fig. 1 in which the cell viability was calculated in comparison with H2O2-treated group. It should be noted that compound 4c did not show any toxicity at the tested concentrations. Based on the results, compound 4c remarkably increased the viability of H2O2-treated cells from about 50% to 83% at 10 μM concentrations. In general, compound 4c significantly protected neurons against cell death in all used concentrations (P value <0.001) (see Fig. 2).

![Scheme 1. Synthesis of compounds 4a–o: (a) NH4SCN, acetone, reflux, 10–20 min; (b) acetone, reflux, 40–60 min.](image-url)
showed the highest antioxidant activity, as determined by FRAP assay. The viability assay of the selected compound 4c against oxidative stress-induced cell death in differentiated PC12 cells revealed that compound 4c significantly protected neurons against cell death in low concentrations.

The inhibitors of 15-LOX, such as compound 4c prototype maybe useful for preventing and treating inflammatory diseases such as asthma, psoriasis, osteoarthritis, rheumatoid arthritis, and atherosclerosis. Particularly, the ability of compound 4c to prevent oxidative stress-induced cell death in neurons revealed that it may be applicable to neuroprotection in a variety of neurodegenerative diseases such as stroke where oxidative stress is a major cause of injury. Future studies of this novel neuroprotective inhibitor of 15-LOX, including investigation of their ADMET properties and in vivo efficacy are required to demonstrate the usefulness of the agent to combat neurodegenerative diseases.

4. Experimental

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. 1H- and 13C NMR spectra were recorded on Bruker FT-400 using TMS as an internal standard. The IR spectra were obtained on a Nicolet Magna FTIR 550 spectrometer (KBr disks). Mass spectra were recorded with an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. The elemental analysis was performed with an Elementar Analysensystem GmbH VarioEL CHNS mode.

4.1. General procedure for the synthesis of compounds 4a–o

A solution of aroyl chloride 1 (1 mmol) and ammonium thiocyanate (1 mmol) in acetone (8 mL) was heated under reflux for 10–20 min. After completion of reaction (checked by TLC), the reaction mixture was cooled to room temperature and the formed precipitate (NH4Cl) was filtered off. To the freshly prepared solution of benzoyl isothiocyanate derivative 2, sulfanilamide 3 (1 mmol) was added and the mixture was stirred under reflux for 40–60 min. Upon completion of reaction (checked by TLC), the resulting precipitate was collected by filtration and recrystallized from EtOH to give the pure product 4.

4.1.1. N-((4-Sulfamoylphenyl)carbamothioyl)benzamide (4a)

Yield: 75%, mp 205–207 °C. IR (KBr): 3369, 3340, 3264, 1663, 1602, 1336, 1157 cm−1. 1H NMR (400 MHz, DMSO-d6): 12.72 (s, 1H, NH), 11.71 (s, 1H, NH), 7.99 (dd, J = 7.7, 1.2 Hz, 2H, H3, H5), 7.91 (d, J = 8.5 Hz, 2H, sulfamoylphenyl), 7.86 (d, J = 8.5 Hz, 2H, sulfamoylphenyl), 7.66 (t, J = 7.7 Hz, 1H, H4), 7.55 (t, J = 7.7 Hz, 2H, H3, H5), 7.42 (s, 2H, NH2), 13C NMR (100 MHz, DMSO-d6): 179.9, 168.7, 141.4, 133.7, 132.5, 129.2, 128.9, 126.7, 126.0, 124.8, 20.0. MS (70 eV): m/z = 335.04 [M+]. Anal. Calcd for C14H13N3O3S2: C, 51.56; H, 3.91; N, 12.63. Found: C, 50.30; H, 4.21; N, 12.36.

4.1.2. 2-Methyl-N-((4-sulfamoylphenyl)carbamothioyl)benzamide (4b)

Yield: 70%, mp 203–205 °C. IR (KBr): 3406, 3292, 1687, 1586, 1522, 1330, 1152 cm−1. 1H NMR (400 MHz, DMSO-d6): 12.66 (s, 1H, NH), 11.85 (s, 1H, NH), 7.93 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.85 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.52 (d, J = 7.6 Hz, 1H, H4), 7.45 (dt, J = 7.6, 1.2 Hz, 1H, H3), 7.42 (s, 2H, NH2), 7.31 (m, 2H, H3, H5), 2.43 (s, 3H, CH3). 13C NMR (100 MHz, DMSO-d6): 179.7, 168.7, 141.7, 141.3, 136.6, 134.3, 131.5, 131.1, 128.7, 126.7, 124.8, 20.0. MS (70 eV): m/z = 349.06 [M+]. Anal. Calcd for C15H15N3O3S2: C, 51.56; H, 4.33; N, 12.03. Found: C, 51.38; H, 4.49; N, 11.87.
4.1.3. 3-Methyl-N-((4-sulfamoylphenyl)carbamothioyl)benzamide (4c)

Yield: 77%, mp 209–211 °C. IR (KBr): 3360, 3310, 3011, 1665, 1592, 1524, 1330, 1154 cm⁻¹. 1H NMR (400 MHz, DMSO-d₆): 12.74 (s, 1H, NH), 11.64 (s, 1H, NH), 7.91 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.86 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.84 (d, J = 3.2 Hz, H₂, H₃), 7.78 (d, J = 7.6 Hz, 1H, H₄), 7.48 (d, J = 7.6 Hz, 1H, H₅), 7.44 (t, J = 7.6 Hz, 1H, H₆), 7.42 (s, 2H, NH₂), 2.40 (s, 3H, CH₃). 13C NMR (100 MHz, DMSO-d₆): 179.7, 168.7, 141.8, 141.8, 134.8, 134.5, 129.6, 128.9, 126.7, 126.4, 124.8, 21.3. MS (70 eV): m/z = 349.06 [M⁺]. Anal. Calcld for C₁₅H₁₃N₂O₂S₂: C, 51.56; H, 4.33; N, 12.03. Found: C, 51.72; H, 4.53; N, 11.81.

4.1.4. 4-Methyl-N-((4-sulfamoylphenyl)carbamothioyl)benzamide (4d)

Yield: 77%, mp 215–217 °C. IR (KBr): 3354, 3255, 2995, 1669, 1598, 1525, 1330, 1154 cm⁻¹. 1H NMR (400 MHz, DMSO-d₆): 12.76 (s, 1H, NH), 11.56 (s, 1H, NH), 7.93–7.90 (m, 4H, sulfamoylphenyl), 7.85 (d, J = 8.4 Hz, 2H, H₂, H₃), 7.42 (s, 2H, NH₂), 7.36 (d, J = 8.4 Hz, 2H, H₂, H₃), 2.40 (s, 3H, CH₃). 13C NMR (100 MHz, DMSO-d₆): 179.9, 168.5, 141.7, 141.4, 129.3, 129.3, 126.7, 124.8, 12.02, 21.8. MS (70 eV): m/z = 349.06 [M⁺]. Anal. Calcld for C₁₅H₁₃N₂O₂S₂: C, 51.56; H, 4.33; N, 12.03. Found: C, 51.41; H, 4.19; N, 12.20.

4.1.5. 4- méthoxy-N-((4-sulfamoylphenyl)carbamothioyl)benzamide (4e)

Yield: 75%, mp 202–204 °C. IR (KBr): 3369, 3345, 3193, 1673, 1599, 1531, 1340, 1159 cm⁻¹. 1H NMR (400 MHz, DMSO-d₆): 12.85 (s, 1H, NH), 11.53 (s, 1H, NH), 8.03 (d, J = 8.8 Hz, 2H, H₂, H₃), 7.91 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.86 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.41 (s, 2H, NH₂), 7.08 (d, J = 8.8 Hz, 2H, H₂, H₃), 3.86 (s, 3H, OCH₃). 13C NMR (100 MHz, DMSO-d₆): 180.0, 1679, 163.8, 141.7, 141.4, 131.5, 126.7, 124.8, 124.2, 114.3, 56.1. MS (70 eV): m/z = 365.05 [M⁺]. Anal. Calcld for C₁₅H₁₃F₂N₂O₂S₂: C, 49.30; H, 4.14; N, 11.50. Found: C, 49.18; H, 3.98; N, 11.28.

4.1.6. 4-Nitro-N-((4-sulfamoylphenyl)carbamothioyl)benzamide (4f)

Yield: 80%, mp 203–205 °C. IR (KBr): 3350, 3267, 1682, 1593, 1529, 1339, 1157 cm⁻¹. 1H NMR (400 MHz, DMSO-d₆): 12.49 (s, 1H, NH), 12.07 (s, 1H, NH), 8.35 (d, J = 8.8 Hz, 2H, H₂, H₃), 8.17 (d, J = 8.8 Hz, 2H, H₂, H₃), 7.91–7.85 (m, 4H, sulfamoylphenyl), 7.42 (s, 2H, NH₂). 13C NMR (100 MHz, DMSO-d₆): 179.6, 168.9, 150.3, 142.2, 141.9, 140.9, 130.8, 126.8, 124.9, 123.9. MS (70 eV): m/z = 380.02 [M⁺]. Anal. Calcld for C₁₅H₁₃N₂O₂S₂: C, 44.21; H, 3.18; N, 14.73. Found: C, 44.40; H, 3.33; N, 14.58.

4.1.7. 3-Fluro-N-((4-sulfamoylphenyl)carbamothioyl)benzamide (4g)

Yield: 77%, mp 190–192 °C. IR (KBr): 3417, 3322, 3244, 3030, 1676, 1605, 1595, 1327, 1155 cm⁻¹. 1H NMR (400 MHz, DMSO-d₆): 12.45 (s, 1H, NH), 11.86 (s, 1H, NH), 7.90 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.85 (d, J = 8.8 Hz, 2H, sulfamoylphenyl), 7.73–7.70 (m, 1H, H₄), 7.68–7.63 (m, 1H, H₅), 7.42 (s, 2H, NH₂), 7.40–7.31 (m, 2H, H₂, H₃). 13C NMR (100 MHz, DMSO-d₆): 179.3, 165.7, 159.8 (d, Jₚ₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋˓→
4.11.4  N-([4-Sulfa&mymophenyl]carbamothioyl)furan-2-carboxamide (4n)

Yield: 72%, mp 217–219 °C. IR (KBr): 3335, 3255, 1654, 1589, 1536, 1341, 1135 cm⁻¹. 1H NMR (400 MHz, DMSO-d₆): 12.56 (s, 1H, NH), 11.75 (s, 1H, NH), 8.40 (d, J = 4.5 Hz, 1H, thiophene), 8.07 (d, J = 4.5 Hz, 1H, thiophene), 7.85 (d, J = 6.4 Hz, 2H, sulfamoylphenyl), 7.83 (d, J = 5.4 Hz, 2H, sulfamoylphenyl), 7.40 (s, 2H, NH₂), 7.27 (t, J = 4.5 Hz, 1H, thiophene). 13C NMR (100 MHz, DMSO-d₆): 179.5, 162.4, 141.8, 141.4, 137.0, 136.0, 129.3, 129.7, 124.9. MS (70 eV): m/z = 325.02 [M+]. Anal. Calcd for C₁₂H₁1N₃O₃S₃: C, 44.30; H, 3.28; N, 13.15. Found: C, 44.18; H, 3.28; N, 13.15.

4.11.5  N-([4-Sulfa&mymophenyl]carbamothioyl)thiophene-2-carboxamide (40)

Yield: 72%, mp 217–219 °C. IR (KBr): 3335, 3255, 1654, 1589, 1536, 1341, 1135 cm⁻¹. 1H NMR (400 MHz, DMSO-d₆): 12.56 (s, 1H, NH), 11.75 (s, 1H, NH), 8.40 (d, J = 4.5 Hz, 1H, thiophene), 8.07 (d, J = 4.5 Hz, 1H, thiophene), 7.84 (d, J = 6.4 Hz, 2H, sulfamoylphenyl), 7.83 (d, J = 4.5 Hz, 2H, sulfamoylphenyl), 7.40 (s, 2H, NH₂), 7.27 (t, J = 4.5 Hz, 1H, thiophene). 13C NMR (100 MHz, DMSO-d₆): 179.5, 162.4, 141.8, 141.4, 137.0, 136.0, 129.3, 129.7, 124.9. MS (70 eV): m/z = 341.00 [M⁺]. Anal. Calcd for C₁₂H₁₁N₃O₄S₂: C, 44.18; H, 3.28; N, 12.92. Found: C, 44.18; H, 3.28; N, 13.15.

4.2  15-LOX inhibition assay

The stock solution of tested compounds was prepared in DMSO (1 mL) and phosphate buffer (9 mL, 0.1 M, pH = 8). This stock solution was added to test solution containing enzyme (1 mL) and phosphate buffer (9 mL, 0.1 M, pH = 4.2). 15-LOX inhibition assay

4.3  Cell culture, differentiation, and viability assay

The neuroprotective activity of the selected compound 4c against oxidative stress-induced cell death was evaluated in differentiated PC12 cells. Firstly, rat undifferentiated PC12 cells were cultured in RPMI 1640 media with 10% FCS containing 100 units/mL penicillin and 100 µg/mL streptomycin (All from Gibco, Grand Island, NY, USA) and then the reported protocol was followed to obtaining differentiated PC12 cells [28]. Differentiated PC12 cells were incubated with different concentrations (1, 5 and 10 µM) of the compounds for 3 h before treatment with H₂O₂ (300 µM). The cell viability was determined after 24 h by using the MTT assay as reported method [29].

4.4  FRAP assay

The target compounds were evaluated for their total antioxidant activity using FRAP assay [26]. In this method the colorless [Fe(III)-TPTZ (2,4,6-Tris(2-pyridyl)-S-triazine)] complex is reduced to colored [Fe(II)-TPTZ] complex by the compounds. To 3 mL of FRAP reagent (10 mM TPTZ and 20 mM FeCl₃ in 300 mM acetate buffer (pH = 3.6), 100 mL of compounds solution was added. Being incubated at 37 °C for 15 min, the change of absorbance was measured at 585 nm and the concentration of the reduced Fe(II) was calculated according to the calibration curve of ferrous sulfate (FeSO₄) as standard. The antioxidant activity was expressed as mmol Fe(II) per gram of dry mass of compounds. Data are mean of three independent experiments and are compared to ascorbic acid as reference.

4.5 Molecular modeling study

All docking studies were performed using Autodock Vina (ver. 1.1.1) [30]. For this purpose, the crystal structure of soybean lipooxygenase complexed with 13(S)-hydroxy-9(Z)-2,11(E)-octadecadienoic acid (code ID: 1IK3) were retrieved from protein data bank. Then, the co-crystallized ligand and water molecules were removed and the protein was converted to pdbqt format using Autodock Tools (1.5.4) [31]. The 2D structures of ligands were sketched using MarvinSketch 5.8.3, 2012, ChemAxon (http://www.chemaxon.com) and then converted to 3D and pdbqt format by Openbabel (ver. 2.3.1) [32]. The docking parameters were set as follow: size_x = 20; size_y = 20; size_z = 20; center_x = 19.693; center_y = 0.054; center_z = 17.628; exhaustiveness = 100; num_modes = 15. The other parameters were left as default. Finally, the conformations with the most favorable free energy of binding were selected for analyzing the interactions between the target enzyme and inhibitors. All the 3D models are generated using the Chimera 1.6 software [33].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.054.

References


