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Original Research Article

Design and Synthesis of Novel Tetrapeptide Analogues as New Cytotoxic Agents

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HIGHLIGHTS

- A group of tripeptides was reported as COX-2 inhibitors with antiproliferative activity.
- New tetrapeptides containing methyl sulfonyl group at the para position of a phenyl ring were synthesized.
- Some of novel compounds exhibited more potent cytotoxic effect than Celecoxib as the reference.

ABSTRACT

New series of compounds based on a tetrapeptide scaffold containing methyl sulfonyl group at the para position of a phenyl ring were synthesized and their cytotoxic activities were examined against several human cancer cell lines including MCF-7 (breast cancer Cell Line), HepG2 (human liver cancer Cell Line), HT-29 (Human Colorectal Adenocarcinoma Cell Line) and A549 (adenocarcinomic human alveolar basal epithelial cells) using MTT assay. Based on the results, among the synthesized peptides, 5e, 5f, 1g, and 3g were the most potent cytotoxic compounds that were more toxic than the reference compound, Celecoxib, against the tested cell lines. These compounds could be candidate for finding cytotoxic agents with new peptide scaffolds which show COX-2 inhibitory activity as well.

Keywords:
Cytotoxic activity
Methyl sulfonyl group
MTT assay
Synthesis
Tetrapeptide scaffold

Introduction

Cancer is known as an unrestrained division of cells with invasion to other tissues, producing vascularization, tumor lumps which may spread to all parts of the body. Cyclooxygenases (COXs) are essential enzymes in conversion of arachidonic acid to prostaglandins. COX-1 is expressed in various tissues and plays some protective roles in digestive system, renal organ, and homeostasis. COX-2 enzyme isoform is expressed only when pathogenic conditions have been occurred and therefore inflammatory process is initiated by this enzyme (Vane et al., 1998; McAdam et al., 1999). There is a diversity of mechanisms which involve in tumor growth inhibition. These mechanisms include restriction of gene expression, angiogenesis, and signal transduction pathways, etc. Another way of anti-cancer peptides to show therapeutic activity is through binding to specific receptors such as COX-2 enzymes (Yang et al., 1998; Chell et al., 2006). COX-2 is assumed to be expressed at great levels in various types of cancer cells, but not in normal tissues. It has been proved that when COX-2 is overexpressed, then PGE_2 increases in cancer (Koki and Masferrer, 2002; Li et al., 2002) which prompts to develop metastatic invasion of tumor cells (Ye et al., 2004) . These findings have been verified by the antiproliferative activity of Celecoxib as a known potent and selective inhibitor of COX-2 (Kang et al., 2000; Thundimadathil, 2012). In one study

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some tripeptides were reported as COX-2 inhibitors. The tripeptides were checked by *in-vitro* experiments using surface plasmon resonance (SPR) technique. Among the tripeptides, one was recognized to be as a promising lead for another class of COX-2 inhibitors (Al Houari et al., 2008). Another study reported a series of fluorobenzoylated di- and tripeptides which showed COX-2 inhibitory action compared to Celecoxib (Najim et al., 2010). A recent study reported a series of tripeptides as COX-2 inhibitors in relation to indomethacin and diclofenac. In such study, the COX inhibitory activity of all 203 possible natural tripeptide sequences was tested. Based on the data acquired from virtual screening, just those peptides with better affinity were chosen which demonstrated strong recognition of COX-2 whereas indicating a lower interaction towards COX- 1 (Somvanshi et al., 2007). In recent years, peptides have been considered as therapeutic candidates in the treatment of various diseases such as cancer. Peptides can target cancer cells without disturbing normal cells (Sharma et al., 2012).

The aim of this study is to design, synthesize, and examine some new tetrapeptide analogues of the Cox-2 inhibitors expected to exhibit anti-cancer activity as well. For designing the new modified tetrapeptides, an acidic amino acid such as aspartic acid was chosen to be attached to an aromatic amino acid (i.e.,phenylalanine, tyrosine, tryptophan or histidine), then to be connected to a linear amino acid (i.e., glycine, alanine, valine, isoleucine and serine) and ended with a moiety containing a methyl sulfonyl group at the *para* position of a phenyl ring as a pharmacophoric entity characterized of Cox-2 inhibitors' scaffold. The cytotoxic activities of synthesized peptides were evaluated against various human cancer cell lines including MCF-7, HepG2, HT-29, and A549.

Materials and Methods

General

*N*α -Fmoc-protected amino acids, Wang resin were from Bachem, Swithzerland. HOBt, DIC, piperazine, and trifluoroacetic acid were purchased (from Sigma Aldrich, Italy). Peptide synthesis solvents, reagents, were analytical grade and acquired from commercial source (Merck, Germany) and used without further purification, otherwise noted. Infrared spectra were acquired on a Perkin-Elmer 1420 ratio recording spectrometer. A Bruker FT-400 MHz instrument (Brucker Biosciences, USA) was used to acquire 1HNMR spectra; DMSO-d6 was used as solvent. Coupling constant (J) values were estimated in hertz (Hz) and spin multiples were given as s (singlet), d (double), t (triplet), q (quartet), m (multiplet), and br (broad). The mass spectral measurements were performed on a 6410 Agilent LCMS triple quadrupole mass spectrometer (LCMS) with an electrospray ionization (ESI) interface.

General procedure for attaching the first amino acid

The synthesis of modified tetrapeptides (1e-5h) was carried out according to the solid phase approach using standard Fmoc methodology in a manual reaction vessel. The first amino acid, Fmoc-Xaa-OH, was linked onto the Wang resin (100–200 mesh, 1% DVB, 1 mmol/g) using HOBt (2 eq) and DIC (1 eq) as activating agents and a catalytic amount of DMAP. The *N*α-Fmoc protecting group was removed by treating amino acid-resin with a 10% solution of piperazine in DMF (30 min) and then the resin was washed with DMF (5×).

General procedure for the preparation of modified tetrapeptides (1e-5h)

The following reactant materials, Fmoc-amino acids (2 eq, each), DIC (2 eq), HOBt (2 eq) were dissolved in DMF or DCM and added to the resin and shaken slowly. The coupling time lasted 2 hours. The peptide-resin was washed with DMF (3×) and then the N^{α}-Fmoc protecting groups were removed by treating the protected peptide- resin with a 10% solution of piperazine in DMF (30 min), followed by washing with DMF (5×). The coupling process was repeated for attaching 4-(Methylsulfonyl) benzoic acid, at the end. The completed peptide- resin was washed with DMF (3×) and DCM (3×), and methanol (3×). The peptides were final deprotected and cleaved from the solid support with trifluoroacetic acid/DCM/anisole /triisopropyl-silane (50: 45: 2.5: 2.5) for 2 h. The resin was filtered off and the crude peptide was precipitated by adding cold diethyl ether and washed with diethyl ether. The residual ether was removed by evaporation and the product was lyophilized.

General procedure for the preparation of 4-(Methylsulfonyl) benzoic acid

4-(Methylthio) benzaldehyde (3 mL) was dissolved in THF (10 mL) to which, Oxone (10 g in 30 mL THF/water) was added. The mixture was stirred at room temperature for 24 h. After evaporation of THF, the residue was extracted with chloroform, washed with 10% aqueous sodium bicarbonate and dried with anhydrous sodium sulfate and then the solvent was evaporated. In the most cases, off-white to pale yellow solid was formed. Yield: (70-94%).

Chemistry

p-$MeSO_2$Bz-Gly-Tyr-Asp (1e)

Yield: 78%; White solid; IR (KBr): ν (cm^{-1}) 1737,

1732(C=O); 1305, 1161 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 2.55-2.62 (m, 2H, CH_2, aspartic acid), 2.70-2.81 (m, 2H, CH_2, benzyl), 3.2 (s, 3H, SO_2CH_3), 3.80 (s, 2H, CH_2, Gly), 3.83-3.86 (d, 1H, CH), 3.93-4.1 (d, 1H, CH), 4.60 (s, 1H, phenol), 7.31-7.33 (d, *J*=7.8 Hz, 2H, phenol H_3&H_5), 7.62-7.64 (d, 2H, *J*=7.8 Hz phenol H_2&H_6), 8.02-8.04 (d, 2H, *J*=8.6 Hz, 4-methylsulfonylphenyl H_2&H_6), 8.06-8.08 (d, *J*=8.6 Hz, 2H, 4-methylsulfonylphenyl H_3&H_5), 8.16-8.19 (d, 1H, NH), 8.48-8.5 (d, 1H, NH), 8.95-8.98 (d, 1H, NH), 10.83 (s, 1H, COOH), 12.4 (br, 1H, COOH); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 28.3, 36.4, 43.2 (CH_2), 40.1 (CH_3), 43.5, 53.6 (CH), 110.3, 115.5, 118.6, 121.2, 127.4, 130.1, 136.4, 143.4 (C–Ar), 165.6, 168.8, 171.8 (CONH), 172.1, 172.8 (COOH)ppm; LC-MS (ESI) *m/z* = 535 (M-1).

p-$MeSO_2$Bz -Val-Tyr-Asp (2e)

Yield: 81%; White solid; IR (KBr): ν (cm^{-1}) 1737, 1732(C=O); 1305, 1161(SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 1.03-1.06 (d, 6H, CH_3), 2.55-2.62 (m, 2H, CH_2, aspartic acid), 2.60 (d, CH, ipr), 2.70-2.81 (m, 2H, CH_2, benzyl), 3.2 (s, 3H, SO_2CH_3), 4.26-4.37 (d, 1H, CH), 4.60 (s, 1H, phenol), 4.63-4.75 (m, 1H, CH), 4.77-4.79 (d, 1H, CH), 7.31-7.33 (d, *J*=7.8 Hz, 2H, phenol H_3&H_5), 7.62-7.64 (d, 2H, *J*=7.8 Hz phenol H_2&H_6), 8.02-8.04 (d, 2H, *J*=8.6 Hz, 4-methylsulfonylphenyl H_2&H_6), 8.06-8.08 (d, *J*=8.6 Hz, 2H, 4-methylsulfonylphenyl H_3&H_5), 8.16-8.19 (d, 1H, NH), 8.48-8.5 (d, 1H, NH), 8.95-8.98 (d, 1H, NH), 10.83 (s, 1H, COOH), 12.4 (br, 1H, COOH);^{13}C NMR (100.6 MHz, DMSO-d6) δ = 36.3, 38.3 (CH_2), 19.0, 43.5 (CH_3), 30.4, 49, 53.8, 59.7 (CH), 126.6, 127.3, 128.3, 129.0, 129.6, 138.0, 139.2, 143.3 (C–Ar), 158.5, 165.6, 170.8 (CONH), 171.0, 172.0 (COOH) ppm; LC-MS (ESI) *m/z* = 576.1 (M-1).

p-$MeSO_2$Bz -Ile-Tyr-Asp (3e)

Yield: 79%; White solid; IR (KBr): ν (cm^{-1}) 1737, 1732 (C=O); 1305, 1161 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 0.84-0.86 (t, 3H, CH_3), 1.08 (d, 3H, CH_3), 1.32 (m, 2H, CH_2), 2.23 (m, 1H, CH), 2.55-2.62 (m, 2H, CH_2, aspartic acid), 2.70-2.81 (m, 2H, CH_2, benzyl), 3.2 (s, 3H, SO_2CH_3 4.26-4.37 (d, 1H, CH), 4.60 (s, 1H, phenol), 4.63-4.75 (m, 1H, CH), 4.77-4.79 (d, 1H, CH), 7.31-7.33 (d, *J*=7.8 Hz, 2H, phenol H_3&H_5), 7.62-7.64 (d, 2H, *J*=7.8 Hz phenol H_2&H_6), 8.02-8.04 (d, 2H, *J*=8.6 Hz, 4-methylsulfonylphenyl H_2&H_6), 8.06-8.08 (d, *J*=8.6 Hz, 2H, 4-methylsulfonylphenyl H_3&H_5), 8.16-8.19 (d, 1H, NH), 8.48-8.5 (d, 1H, NH), 8.95-8.98 (d, 1H, NH), 10.83 (s, 1H, COOH), 12.4 (br, 1H, COOH); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 25.0, 28.1, 29.8 (CH_2), 11.0, 15.6, 44.4 (CH_3), 26.4, 51.8, 53.9, 58.5 (CH), 126.6, 127.0, 128.4, 129.0, 137.9, 139.1, 143.4, 139.2, 143.3 (C–Ar), 165.6, 171.0, 172.2 (CONH), 173.0, 173.3 (COOH) ppm; LC-MS (ESI) *m/z* = 576.2 (M-1).

p-$MeSO_2$Bz -Ala-Tyr-Asp (4e)

Yield: 85%; White solid; IR (KBr): ν (cm^{-1})1737, 1732(C=O); 1305, 1161(SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 1.34 (d, 3H, CH_3), 2.55-2.62 (m, 2H, CH_2, aspartic acid), 2.70-2.81 (m, 2H, CH_2, benzyl), 3.2 (s, 3H, SO_2CH_3), 4.39-4.46 (d, 1H, CH), 4.51-4.63 (q, 1H, CH), 4.60 (s, 1H, phenol), 4.64-4.67 (d, 1H, CH), 7.31-7.33 (d, *J*=7.8 Hz, 2H, phenol H_3&H_5), 7.62-7.64 (d, 2H, *J*=7.8 Hz phenol H_2&H_6), 8.02-8.04 (d, 2H, *J*=8.6 Hz, 4-methylsulfonylphenyl H_2&H_6), 8.06-8.08 (d, *J*=8.6 Hz, 2H, 4-methylsulfonylphenyl H_3&H_5), 8.16-8.19 (d, 1H, NH), 8.48-8.5 (d, 1H, NH), 8.95-8.98 (d, 1H, NH), 10.83 (s, 1H, COOH), 12.4 (br, 1H, COOH); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 36.4, 39.3 (CH_2), 18.0, 43.9 (CH_3), 49.0, 49.5, 54.3 (CH), 115.2, 127.3, 128.9, 138.9, 139.2, 143.4 (C–Ar), 156.2, 165.3, 171.3 (CONH), 172.1, 172.7 (COOH) ppm; LC-MS (ESI) *m/z* = 548.1 (M-1).

p-$MeSO_2$Bz -Ser-Tyr-Asp (5e)

Yield: 67%; White solid; IR (KBr): ν(cm^{-1}) 1737, 1732(C=O); 1305, 1161 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 2.12 (s, 1H, OH), 2.55-2.62 (m, 2H, CH_2, aspartic acid), 2.70-2.81 (m, 2H, CH_2, benzyl), 3.2 (s, 3H, SO_2CH_3), 4.26-4.37 (d, 1H, CH), 4.60 (s, 1H, phenol), 4.63-4.75 (m, 1H, CH), 4.77-4.79 (d, 1H, CH), 7.31-7.33 (d, *J*=7.8 Hz, 2H, phenol H_3&H_5), 7.62-7.64 (d, 2H, *J*=7.8 Hz phenol H_2&H_6), 8.02-8.04 (d, 2H, *J*=8.6 Hz, 4-methylsulfonylphenyl H_2&H_6), 8.06-8.08 (d, *J*=8.6 Hz, 2H, 4-methylsulfonylphenyl H_3&H_5), 8.16-8.19 (d, 1H, NH), 8.48-8.5 (d, 1H, NH), 8.95-8.98 (d, 1H, NH), 10.83 (s, 1H, COOH), 12.4 (br, 2H, COOH); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 31.1, 39.3, 61.8 (CH_2), 40.1 (CH_3), 43.7, 49.1, 54.0 (CH), 125.1, 127.2, 128.4, 128.9, 137.9, 138.9, 139.2, 143.3 (C–Ar), 158.5, 165.6, 171.1(CONH), 172.0, 172.7 (COOH) ppm; LC-MS (ESI) *m/z* = 564.1 (M-1).

p-$MeSO_2$Bz -Gly-Phe-Asp (1f)

Yield: 76%; White solid; IR (KBr): ν(cm-1)1737, 1732(C=O); 1305, 1161 (SO2); 1HNMR (400 MHz, DMSO-d6): δ ppm 2.63-2.74 (m, 2H, CH2, aspartic acid), 2.98-3.17 (m, 2H, CH2, benzyl), 3.2 (s, 3H, SO_2CH_3), 4.1 (s, 2H, CH_2, Gly), 4.63-4.75 (m, 1H, CH), 4.77-4.79 (d, 1H, CH), 7.14-7.18 (t, 1H, Phenyl H_4), 7.18-7.20 (d, 2H, J=7 Hz phenyl H_2&H_6), 7.23-7.25 (d, 2H, J=7 Hz phenyl H_3&H_5), 8.03-8.05 (d, 2H, J=8, 4-methylsulfonylphenyl H_2-H_6), 8.08-8.10 (d, 2H, J=8, 4-methyl sulfonylphenyl H_3-H_5), 8.30-8.33 (d, 1H, NH), 8.43-8.45 (d, 1H, NH), 8.69-8.71 (d, 1H, NH), 12.6 (br, 2H, COOH); ^{13}C NMR

(100.6 MHz, DMSO-d6) δ = 28.3, 36.4, 43.2 (CH_2), 40.1 (CH_3), 43.5, 53.6 (CH), 110.3, 115.5, 118.6, 121.2, 127.4, 130.1, 136.4, 143.4 (C–Ar), 165.6, 168.8, 171.8 (CONH), 172.1, 172.8 (COOH) ppm; LC-MS (ESI) *m/z* = 518.1 (M-1).

p-$MeSO_2$Bz -Val-Phe-Asp (2f)

Yield: 78%; White solid; IR(KBr): ν (cm^{-1}) 1731, 1740 (C=O);1324, 1154 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 1.03-1.06 (d, 6H, CH_3), 2.63-2.74 (m, 2H, CH_2, aspartic acid), 2.60 (d, CH, ipr), 2.98-3.17 (m, 2H, CH_2, benzyl), 3.2 (s, 3H, SO_2CH_3), 4.26-4.37 (d, 1H, CH), 4.63-4.75 (m, 1H, CH), 4.77-4.79 (d, 1H, CH), 7.14-7.18 (t, 1H, Phenyl H_4), 7.18-7.20 (d, 2H, J=7 Hz phenyl H_2&H_6), 7.23-7.25 (d, 2H, J=7 Hz phenyl H_3&H_5), 8.03-8.05 (d, 2H, J=8, 4-methylsulfonylphenyl H_2-H_6), 8.08- 8.10 (d, 2H, J=8, 4-methylsulfonylphenyl H_3-H_5), 8.30-8.33 (d, 1H, NH), 8.43-8.45 (d, 1H, NH), 8.69-8.71 (d, 1H, NH), 12.6 (br, 2H, COOH); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 36.3, 38.3(CH_2), 19.0, 43.5 (CH_3), 30.4, 49, 53.8, 59.7 (CH), 126.6, 127.3, 128.3, 129.0, 129.6, 138.0, 139.2, 143.3 (C–Ar), 158.5, 165.6, 170.8 (CONH), 171.0, 172.0 (COOH) ppm; LC-MS (ESI) *m/z* = 560.2 (M-1).

p-$MeSO_2$Bz -Ile-Phe-Asp (3f)

Yield: 81%; White solid; IR (KBr): ν (cm^{-1}) 1731, 1740 (C=O);1324, 1154 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 0.84-0.86 (t, 3H, CH_3), 1.08 (d, 3H, CH_3), 1.32 (m, 2H, CH_2), 2.23 (m, 1H, CH), 2.63-2.74 (m, 2H, CH_2, aspartic acid), 2.98-3.17 (m, 2H, CH_2, benzyl), 3.2 (s, 3H, SO_2CH_3), 4.26-4.37 (d, 1H, CH), 4.63-4.75 (m, 1H, CH), 4.77-4.79 (d, 1H, CH), 7.14-7.18 (t, 1H, Phenyl H_4), 7.18-7.20 (d, 2H, *J*=7 Hz phenyl H_2&H_6), 7.23-7.25 (d, 2H, *J*=7 Hz phenyl H_3&H_5), 8.03- 8.05 (d, 2H, J=8, 4-methylsulfonylphenyl H_2-H_6), 8.08-8.10 (d, 2H, J=8, 4-methylsulfonylphenyl H3-H5), 8.30-8.33 (d, 1H, NH), 8.43-8.45 (d, 1H, NH), 8.69-8.71 (d, 1H, NH), 12.6 (br, 2H, COOH); ^{13}C NMR (100.6 MHz, DMSO-d6) δ =25.0, 28.1, 29.8 (CH_2), 11.0, 15.6, 44.4 (CH_3), 26.4, 51.8, 53.9, 58.5 (CH), 126.6, 127.128.4, 129.0, 137.9, 139.1, 143.4, 139.2, 143.3 (C–Ar), 165.6, 171.0, 172.2 (CONH), 173.0, 173.3 (COOH) ppm ; LC-MS (ESI) *m/z* = 574.1 (M-1).

p-$MeSO_2$Bz -Ala-Phe-Asp (4f)

Yield: 75%; White solid; IR (KBr): ν (cm^{-1}) 1731, 1740 (C=O);1324, 1154 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 1.34 (d, 3H, CH_3), 2.63-2.74 (m, 2H, CH_2, aspartic acid), 2.98-3.17 (m, 2H, CH_2, benzyl), 3.2 (s, 3H, SO_2CH_3), 4.39-4.46(d, 1H, CH), 4.51-4.63 (q, 1H, CH), 4.64-4.67 (d, 1H, CH), 7.14-7.18 (t, 1H, Phenyl H_4), 7.18-7.20 (d, 2H, J=7 Hz phenyl H_2&H_6), 7.23-7.25 (d, 2H, J=7 Hz phenyl H_3&H_5), 8.03-8.05 (d, 2H, J=8, 4-methylsulfonylphenyl H_2-H_6), 8.08- 8.10 (d, 2H, J=8, 4-methylsulfonylphenyl H_3-H_5), 8.30-8.33 (d, 1H, NH), 8.43-8.45(d, 1H, NH), 8.69-8.71 (d, 1H, NH), 12.6(br, 2H, COOH); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 36.4, 39.3 (CH_2), 18.0, 43.9 (CH_3), 49.0, 49.5, 54.3 (CH), 115.2, 127.3, 128.9, 138.9, 139.2, 143.4 (C–Ar), 156.2, 165.3, 171.3(CONH), 172.1, 172.7 (COOH) ppm; LC-MS (ESI) *m/z* = 532.1 (M-1).

p-$MeSO_2$Bz -Ser-Phe-Asp (5f)

Yield: 68%; White solid; IR (KBr): ν (cm^{-1}) 1731, 1740(C=O); 1324, 1154(SO_2); ^{1}HNMR (400MHz, DMSO-d6): δ ppm 2.12 (s, 1H, OH), 2.63-2.74 (m, 2H, CH_2, aspartic acid), 2.98-3.17 (m, 2H, CH_2, benzyl), 3.2 (s, 3H, SO_2CH_3), 3.60-3.90 (m, 2H, CH_2), 4.26-4.37 (d, 1H, CH), 4.63-4.75(m, 1H, CH), 4.77-4.79(d, 1H, CH), 7.14-7.18(t, 1H, Phenyl H_4), 7.19-7.21 (d, 2H, *J*=7 Hz phenyl H_2&H_6), 7.23-7.25 (d, 2H, *J*=7 Hz phenyl H_3&H_5), 8.03-8.05 (d, 2H, J=8, 4-methylsulfonylphenyl H_2-H_6), 8.08-8.10 (d, 2H, J=8, 4-methylsulfonylphenyl H_3-H_5), 8.30-8.33 (d, 1H, NH), 8.43-8.45 (d, 1H, NH), 8.69-8.71 (d, 1H, NH), 12.6 (br, 2H, COOH); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 31.1, 39.3, 61.8 (CH_2), 40.1 (CH_3), 43.7, 49.1, 54.0 (CH), 125.1, 127.2, 128.4, 128.9, 137.9, 138.9, 139.2, 143.3 (C–Ar), 158.5, 165.6, 171.1 (CONH), 172.0, 172.7 (COOH) ppm; LC-MS (ESI) *m/z* = 5483 (M-1).

p-$MeSO_2$Bz -Gly-His-Asp (1g)

Yield: 82%; White solid; IR (KBr): ν (cm^{-1}) 1742(C=O); 1320, 1178 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 2.63-2.74 (m, 2H, CH_2, aspartic acid), 2.63-2.74 (m, 2H, CH_2, imidazole), 3.1 (s, 3H, SO_2CH_3), 4.07 (s, 2H, CH_2 Gly), 4.26-4.27 (d, 1H, CH), 4.77-4.79 (d, 1H, CH), 7.20-7.22 (d, 2H, *J*=10 Hz, 4-methylsulfonylphenyl H_2&H_6), 7.32 (s, 1H, CH, imidazole), 7.87-7.89 (d, *J*=10 Hz, 2H, 4-methylsulfonylphenyl H_3&H_5), 8.38-8.39 (d, 1H, NH), 8.38-8.39 (d, 1H, NH), 8.77-8.78 (d, 1H, NH), 8.93 (s, 1H, CH, imidazole), 12.5 (br, 2H, COOH), 13.79 (s, 1H, NH, imidazole); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 27.9, 36.5, 39.2 (CH_2), 43.7 (CH_3), 49.1, 53.6 (CH) 125.3, 127.4, 128.0, 128.9, 138.1, 138.9, 139.4, 143.2 (C–Ar), 157.1, 163.2, 171.2 (CONH), 171.8, 173.3 (COOH) ppm; LC-MS (ESI) *m/z* = 508.0 (M-1).

p-$MeSO_2$Bz -Val-His-Asp (2g)

Yield: 76%; White solid; IR (KBr): ν (cm^{-1}) 1742(C=O); 1320, 1178 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 0.83-0.86 (d, 6H, CH_3), 2.02 (d, 1H, ipr), 2.50-2.68 (m, 2H, CH_2, aspartic acid), 2.85-3.09 (m, 2H, CH_2, imidazole), 3.1 (s, 3H, SO_2CH_3), 4.12-4.15 (d, 1H, CH), 4.4-4.53 (m, 1H, CH), 4.56-4.68 (d, 1H, CH), 7.93-7.95

(d, 2H, *J*=8.69 Hz, 4-methylsulfonylphenyl H_2&H_6), 7.32 (s, 1H, CH, imidazole), 8.00-8.02 (d, *J*=8.69 Hz, 2H, 4-methylsulfonylphenyl H_3&H_5), 8.15-8.18 (d, 1H, NH), 8.38-8.40 (d, 1H, NH), 8.54-8.63 (d, 1H, NH) , 8.87 (s, 1H, CH, imidazole), 12.5 (br, 2H, COOH), 14.27 (s, 1H, NH, imidazole); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 27.3, 36.3 (CH_2), 19.4, 43.7 (CH_3), 49.0, 51.6, 60.0 (CH), 117.3, 127.3, 129.1, 134.0, 132.7, 139.0, 143.4 (C–Ar), 158.7, 166.1, 170.1 (CONH), 171.4, 172.5 (COOH) ppm; LC-MS (ESI) *m/z* = 550.1 (M-1).

p-MeSO$_2$Bz -Ile-His-Asp (3g)

Yield: 82%; White solid; IR (KBr): 1736, 1704(C=O); 1305, 1141 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 0.84-0.86 (t, 3H, CH_3), 1.08 (d, 3H, CH_3), 1.32 (m, 2H, CH_2), 2.23 (m, 1H, CH), 2.50-2.68 (m, 2H, CH_2, aspartic acid), 2.85-3.09 (m, 2H, CH_2, imidazole), 3.1 (s, 3H, SO_2CH_3), 4.12-4.15 (d, 1H, CH), 4.4-4.53 (m, 1H, CH), 4.56-4.68 (d, 1H, CH), 7.93-7.95 (d, 2H, *J*=8.69 Hz, 4-methyl sulfonylphenyl H_2&H_6), 7.32 (s, 1H, CH, imidazole), 8.00-8.02 (d, *J*=8.69 Hz, 2H, 4-methyl sulfonylphenyl H_3&H_5), 8.15-8.18 (d, 1H, NH), 8.38-8.40 (d, 1H, NH), 8.54-8.63 (d, 1H, NH), 8.87 (s, 1H, CH, imidazole), 12.5 (br, 2H, COOH), 14.27 (s, 1H, NH, imidazole); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 15.7, 25.1, 36.7 (CH_2), 11.0, 11.2, 39.9 (CH_3), 36.3, 49.0, 56.8, 58.2 (CH), 114.9, 117.8, 127.3, 129.0, 129.6, 139.2, 143.3 (C–Ar), 158.6, 165.6, 170.1 (CONH), 171.1, 172.5 (COOH) ppm; LC-MS (ESI) *m/z* = 564.1 (M-1).

p-MeSO$_2$Bz -Ala-His-Asp (4g)

Yield: 68%; White solid; IR (KBr): 1736, 1704(C=O); 1305, 1141 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 1.34 (d, 3H, CH_3), 2.63-2.74 (m, 2H, CH_2, aspartic acid), 2.22-3.04 (m, 2H, CH_2, imidazole), 3.1 (s, 3H, SO_2CH_3), 4.39-4.46 (d, 1H, CH), 4.51-4.63 (q, 1H, CH), 4.64-4.67 (d, 1H, CH), 7.38 (s, 1H, CH, imidazole), 8.02-8.04 (d, 2H, *J*=7.3 Hz, 4-methylsulfonylphenyl H_2&H_6), 8.10-8.12 (d, *J*=7.3 Hz, 2H, 4-methylsulfonylphenyl H_3&H_5), 8.18-8.20 (d, 1H, NH), 8.25-8.27 (d, 1H, NH), 8.36-8.38 (d, 1H, NH), 9.00 (s, 1H, CH, imidazole), 12.5 (br, 2H, COOH), 14.21 (s, 1H, NH, imidazole); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 25.1, 36.4 (CH_2), 17.8, 40.1 (CH_3), 43.7, 49.4, 49.8 (CH), 127.3, 128.9, 129.0, 135.2, 139.0, 143.4, 143.5 (C–Ar), 158.5, 165.2, 172.1 (CONH), 172.4, 172.7(COOH) ppm; LC-MS (ESI) *m/z* = 522.1 (M-1).

p-MeSO$_2$Bz -Ser-His-Asp (5g)

Yield: 77%; White solid; IR (KBr): 1736, 1704(C=O); 1305, 1141 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 2.12 (s, 1H, OH), 2.63-2.74 (m, 2H, CH_2, aspartic acid), 2.22-3.04 (m, 2H, CH_2, imidazole), 3.1 (s, 3H, SO_2CH_3), 3.60-3.90 (m, 2H, CH_2), 4.39-4.46 (d, 1H, CH), 4.51-4.63 (q, 1H, CH), 4.64-4.67 (d, 1H, CH), 7.38 (s, 1H, CH, imidazole), 8.02-8.04 (d, 2H, *J*=7.3 Hz, 4-methylsulfonylphenyl H_2&H_6), 8.10-8.12 (d, *J*=7.3 Hz, 2H, 4-methylsulfonylphenyl H_3&H_5), 8.18-8.20 (d, 1H, NH), 8.25-8.27 (d, 1H, NH), 8.36-8.38 (d, 1H, NH), 9.00 (s, 1H, CH, imidazole), 12.5 (br, 2H, COOH), 14.21 (s, 1H, NH, imidazole); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 40.5, 45.6, 60.1 (CH_2), 40.1 (CH_3), 43.7, 49.0, , 49.8 (CH), 127.3, 128.9, 129.0, 135.2, 139.0, 143.4, 143.5 (C–Ar), 158.6, 165.2, 172.1 (CONH), 172.7, 173.3 (COOH) ppm; LC-MS (ESI) *m/z* = 539 (M-1).

p-MeSO$_2$Bz -Gly-Trp-Asp (1h)

Yield: 67%; White solid; IR (KBr): ν (cm^{-1}) 1738,1727 (C=O); 1305, 1144 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 2.63-2.74 (m, 2H, CH_2, aspartic acid), 2.98-3.17 (m, 2H, CH_2, benzyl), 3.2 (s, 3H, SO_2CH_3), 4.1 (s, 2H, CH2 Gly), 4.63-4.75 (m, 1H, CH), 4.77-4.79 (d, 1H, CH), 7.69 (s, 1H, CH, indole), 8.002-8.005 (m, 4H, indole), 8.02-8.04 (d, 2H, J=8.6 Hz, 4-methylsulfonylphenyl H_2&H_6), 8.10-8.12 (d, J=8.6 Hz, 2H, 4-methylsulfonylphenyl H_3&H_5), 8.38-8.39 (d, 1H, NH), 8.38-8.39 (d, 1H, NH), 8.77-8.78 (d, 1H, NH), 10.5 (s, 1H, NH, indole), 12.4 (br, 2H, COOH); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 27.9, 36.5, 39.2 (CH_2), 43.7 (CH_3), 49.1, 53.6 (CH) 110.6, 111.0, 119.6, 120.2, 125.1, 128.3, 129.9, 137.4, 140.0,143.3 (C–Ar), 158.5, 165.4, 171.5 (CONH), 172.3, 173.5 (COOH) ppm; LC-MS (ESI) *m/z* = 558 (M-1).

p-MeSO$_2$Bz -Val-Trp-Asp (2h)

Yield: 61%; White solid; IR (KBr): ν (cm^{-1}) 1738, 1727 (C=O); 1305, 1144 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 0.83-0.86 (d, 6H, CH_3), 2.02 (d, 1H, ipr), 2.63-2.74 (m, 2H, CH_2, aspartic acid), 2.83-3.05 (m, 2H, CH_2, indole), 3.1 (s, 3H, SO_2CH_3), 4.26-4.27 (d, 1H, CH), 4.5 (m, 1H, CH), 4.77-4.79 (d, 1H, CH), 6.6 (s, 1H, CH, indole), 6.8-7 (m, 4H, indole), 8.02-8.04 (d, 2H, J=8.6 Hz, 4-methylsulfonylphenyl H_2&H_6), 8.10-8.12 (d, J=8.6 Hz, 2H, 4-methylsulfonylphenyl H_3&H_5), 8.38-8.39 (d, 1H, NH), 8.38-8.39 (d, 1H, NH), 8.77-8.78 (d, 1H, NH), 10.5(s, 1H, NH, indole), 12.4 (br, 2H, COOH); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 34.7, 44.4 (CH_2), 19.1, 30.5, 43.7 (CH_3), 49.0, 53.4, 59.6 (CH), 110.3, 111.6, 114.8, 117.7, 118.7, 121.2, 124.0, 127.3, 128.3, 136.3, 139.8, 143.3 (C–Ar), 158.5, 165.8, 170.9 (CONH), 171.7, 172.6 (COOH) ppm; LC-MS (ESI) *m/z* = 599.2 (M-1).

p-MeSO$_2$Bz -Ile-Trp-Asp (3h)

Yield: 70%; White solid; IR (KBr): ν (cm^{-1}) 1738, 1727(C=O); 1305, 1144 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 0.84-0.86 (t, 3H, CH_3), 1.08 (d, 3H,

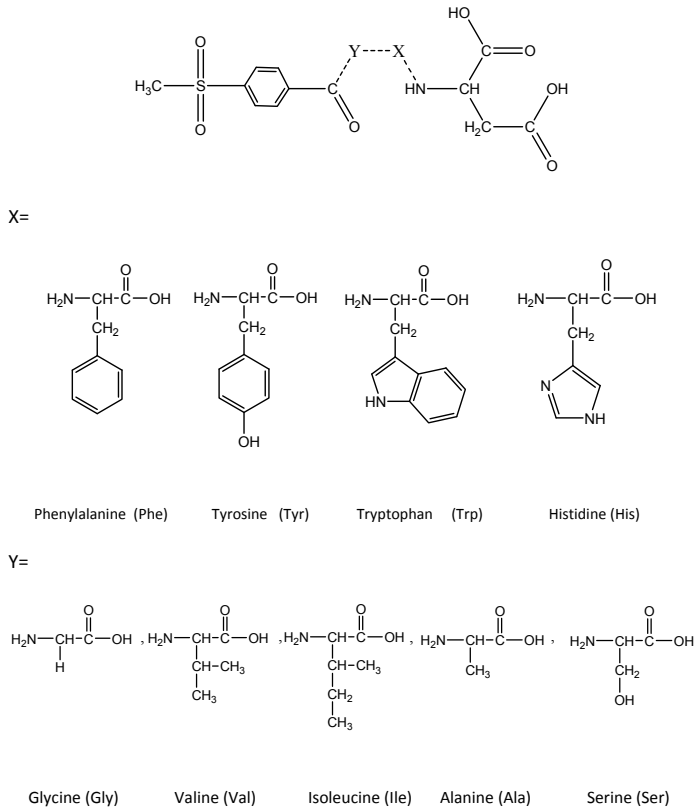


Figure 1. Representative of our designed compounds.

CH_3), 1.32 (m, 2H, CH_2), 2.23 (m, 1H, CH), 2.63-2.74 (m, 2H, CH_2, aspartic acid), 2.83-3.05 (m, 2H, CH_2, indole), 3.1 (s, 3H, SO_2CH_3), 4.26-4.27 (d, 1H, CH), 4.51 (d, 1H, CH), 4.77-4.79 (d, 1H, CH), 6.6 (s, 1H, CH, indole), 6.8-7 (m, 4H, indole), 8.02-8.04 (d, 2H, J=8.6 Hz, 4-methylsulfonylphenyl H_2&H_6), 8.10-8.12 (d, J=8.6 Hz, 2H, 4-methylsulfonylphenyl H_3&H_5), 8.38-8.39 (d, 1H, NH), 8.38-8.39 (d, 1H, NH), 8.77-8.78 (d, 1H, NH), 10.5 (s, 1H, NH, indole), 12.4 (br, 2H, COOH); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 22.3, 27.7, 60.1 (CH_2), 11.6,15.0, 41.7 (CH_3), 24.1, 49.8, 53.7, 65.2 (CH), 106.2, 110.0, 111.7, 115.5, 118.6, 121.3, 127.3, 128.0, 129.6, 130.3 136.5 (C–Ar), 156.2, 168.5, 169.0 (CONH), 171.2, 172.6, (COOH) ppm; LC-MS (ESI) *m/z* = 613.1 (M-1).

p-MeSO$_2$Bz -Ala-Trp-Asp (4h)

Yield: 65%; White solid; IR (KBr): ν (cm^{-1}) 1738, 1727 (C=O); 1305, 1144 (SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 1.34 (d, 3H, CH_3), 2.63-2.74 (m, 2H, CH_2, aspartic acid), 2.83-3.05 (m, 2H, CH_2, indole), 3.1 (s, 3H, SO_2CH_3), 4.26-4.27 (d, 1H, CH), 4.73 (q, 1H, CH), 4.77-4.79 (d, 1H, CH), 6.6 (s, 1H, CH, indole), 6.8-7 (m, 4H, indole), 8.02-8.04 (d, 2H, J=8.6 Hz, 4-methylsulfonylphenyl H_2&H_6), 8.10-8.12 (d, J=8.6 Hz, 2H, 4-methylsulfonylphenyl H_3&H_5), 8.38-8.39 (d, 1H, NH), 8.38-8.39 (d, 1H, NH), 8.77-8.78(d, 1H, NH), 10.5 (s, 1H, NH, indole), 12.4 (br, 2H, COOH); ^{13}C NMR (100.6 MHz, DMSO-d6) δ =27.9, 36.4 (CH_2), 18.1, 39.3 (CH_3), 44.1, 49.0, 53.6 (CH), 110.3, 111.6, 118.6, 121.2, 124.1, 127.3, 128.9, 136.4, 139.0, 143.3 (C–Ar), 158.5, 165.4, 171.7 (CONH), 172.3, 172.7 (COOH) ppm; LC-MS (ESI) *m/z* = 571.1 (M-1).

p-MeSO$_2$Bz -Ser-Trp-Asp (5h)

Yield: 61%; White solid; IR (KBr): ν (cm^{-1}) 1738,1727 (C=O); 1305, 1144(SO_2); ^{1}HNMR (400 MHz, DMSO-d6): δ ppm 2.12 (s, 1H, OH), 2.63-2.74 (m, 2H, CH_2, aspartic acid), 2.83-3.05 (m, 2H, CH_2, indole), 3.1 (s, 3H, SO_2CH_3), 3.85-4.2 (m, 2H, CH_2), 4.26-4.27 (d, 1H, CH), 4.6 (m, 1H, CH), 4.77-4.79 (d, 1H, CH), 6.6 (s, 1H, CH, indole), 6.8-7 (m, 4H, indole), 8.02-8.04 (d, 2H, J=8.6 Hz, 4-methylsulfonylphenyl H_2&H_6), 8.10-8.12 (d, J=8.6 Hz, 2H, 4-methylsulfonylphenyl H_3&H_5), 8.38-8.39 (d, 1H, NH), 8.38-8.39 (d, 1H, NH), 8.77-8.78 (d, 1H, NH), 10.5

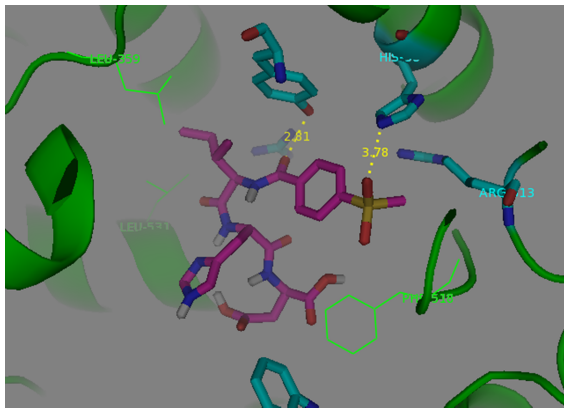


Figure 2. M Docking of 3g in the active site of 6COX. Hydrogen atoms have been removed to improve clarity.

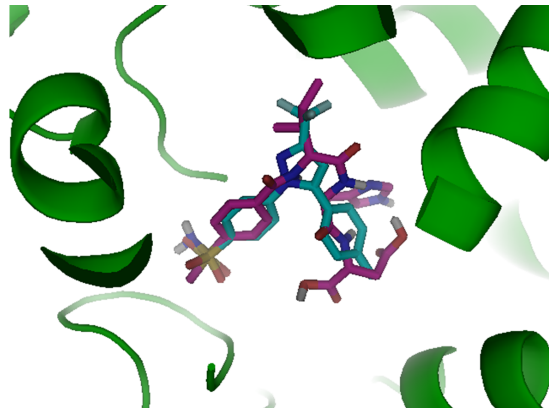


Figure 3. Good superimposition of the modified tetrapeptide compound 3g with celecoxib.

(s, 1H, NH, indole), 12.4 (br, 2H, COOH); ^{13}C NMR (100.6 MHz, DMSO-d6) δ = 29.3, 36.4, 60.1 (CH_2), 43.7 (CH_3), 44.2, 48.0, 53.2 (CH), 111.2, 113.0, 119.1, 122.4, 125.6, 128.1, 131.1, 132.7, 136.3, 137.1, 139.2, 143.3 (C–Ar), 157.1, 165.4, 171.1 (CONH), 172.4, 173.3 (COOH) ppm; LC-MS (ESI) *m/z* = 587.1 (M-1).

Molecular modeling (docking) studies

Docking analysis was operated by autodock vina software (Trott and Olson, 2010). The X-ray crystal structure of the selective COX-2 receptor Celecoxib bound to the human COX-2 active site receptor α was obtained from the RCSB, PDB (6COX) and kollman charge was calculated and non-polar hydrogens were deleted. A grid box of 24-24-24 A˚ with the central X-Y-Z coordinates of X: 23.6652 Y: 23.3127 Z: 47.8268 were determined for calculation of the energy map. For docking validation, Celecoxib was docked in the active site of 6COX with absolutely identical conditions and the docked conformation having minimum docking energy was adjusted to Celecoxib in crystallography with (6COX), applying pymol software.

Cytotoxicity

To determine the cytotoxicity of the modified tetrapeptide derivatives, four human tumor cell lines were used: MCF-7 (breast cancer Cell Line), A549 (adenocarcinoma human alveolar basal epithelial cells), HepG2 (human liver cancer Cell Line), and HT-29 (Human Colorectal Adenocarcinoma Cell Line). Human skin fibroblast cell line was also included for comparison. The cell lines were purchased from Iranian Biological Resource Center (IBRC), Tehran, Iran [18-20]. The cells were grown in RPMI1640 medium at 37 °C under 5% CO_2 enriched with 10% fetal bovine serum (FBS) U/mL penicillin and 100 µg/mL streptomycin. Cell viability was evaluated by using a MTT technique which is based on the transformation of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) dye to purple formazan crystals by mitochondrial succinate dehydrogenase enzyme in alive cells. The cells were cultured into 96-well plates at a concentration of 10^4cells/well and allowed to incubate for 24 h. The cells were incubated with increasing concentrations of the test compounds for 48h. At the end of each analysis period, MTT (10 µL, 5 mg/mL in PBS) was added to each well and the microplate was incubated at 37°C for 4 h. The medium was removed and DMSO (100 µL) was added to each well to liquate the inextricable formazan crystals. Plates were incubated for 30 min at 37°C and the optical densities were read at 570 nm using a spectrophotometer plate reader (Infinite® M200, TECAN)(Mosmann, 1983). Celecoxib was also used as a positive control and DMSO as the solvent of the test compounds. The data are presented as the mean of triplicate number of living cells and IC_{50} was calculated by calibration curve using Prism software.

Results and Discussion

The cytotoxicity activities of products (1e-5h) were determined by their effects on four different cell lines such as A549 (human lung cancer cell line), MCF-7 (breast cancer Cell Line), HT29 (Human Colorectal Adenocarcinoma Cell Line) and HepG2 (human liver cancer Cell Line). To indicate the anti-proliferative activities of the synthesized compounds, the cells were treated with increasing concentrations of synthesized compounds (1–100 µM) and Celecoxib (1–100 µM) as a reference drug. The results of MTT assay are shown in Table 1. The results clearly indicated that modified tetrapeptides (3g and 5f), showed significant cytotoxic

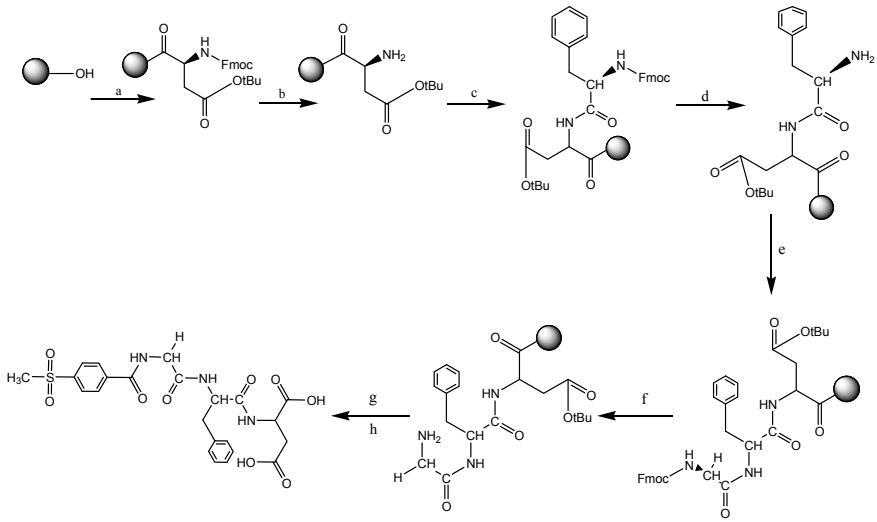
Table 1. *In vitro* antiproliferative activity of compounds **1e-5h** based on MTT assay.

Compounds	X	Y	MCF-7 IC50 (µM)a	HEPG2 IC50 (µM)	HT-29 IC50 (µM)	A549 IC50 (µM)	Human skin fibroblast IC50 (µM)
1e	Tyr	Gly	11.36 ± 0.03	10 ± 0.04	41.98 ± 0.01	10.84 ± 0.08	35.12 ± 0.04
2e	Tyr	Val	10.79 ± 0.21	7.41 ± 0.02	>100	8.87 ± 0.08	85.14 ± 0.03
3e	Tyr	Ile	9.98 ± 0.04	10.38 ± 0.02	29.28 ± 0.02	33.44 ± 0.12	45.13 ± 0.05
4e	Tyr	Ala	>100	>100	>100	9.41 ± 0.11	>100
5e	Tyr	Ser	11.29 ± 0.07	3.30 ± 0.03	11.39 ± 0.03	11.15 ± 0.11	78.16 ± 0.03
1f	Phe	Gly	10.33 ± 0.12	9.47 ± 0.03	37.87 ± 0.02	13.96 ± 0.01	31.15 ± 0.05
2f	Phe	Val	>100	9.08 ± 0.01	10.65 ± 0.01	12.95 ± 0.21	>100
3f	Phe	Ile	13.54 ± 0.01	6.60 ± 0.02	>100	31.92 ± 0.18	48.17 ± 0.04
4f	Phe	Ala	31.44 ± 0.01	>100	31.34 ± 0.02	3.18 ± 0.06	>100
5f	Phe	Ser	9.06 ± 0.03	10.14 ± 0.02	6.756 ± 0.01	3.94 ± 0.14	>100
1g	His	Gly	9.11 ± 0.12	9.22 ± 0.02	10.22 ± 0.01	6.41 ± 0.12	>100
2g	His	Val	11.54 ± 0.09	>100	2.52 ± 0.03	11.26 ± 0.19	>100
3g	His	Ile	2.46 ± 0.03	5.28 ± 0.01	11.01 ± 0.06	11.85 ± 0.08	78.16 ± 0.02
4g	His	Ala	32.72 ± 0.20	8.73 ± 0.02	3.01 ± 0.02	>100	>100
5g	His	Ser	>100	10.86 ± 0.04	8.82 ± 0.03	31.77 ± 0.26	>100
1h	Trp	Gly	9.58 ± 0.05	13.93 ± 0.02	>100	92.68 ± 0.15	81.12 ± 0.01
2h	Trp	Val	29.69 ± 0.16	12.23 ± 0.02	9.34 ± 0.02	31.54 ± 0.05	>100
3h	Trp	Ile	9.66 ± 0.21	>100	31.56 ± 0.02	7.30 ± 0.13	>100
4h	Trp	Ala	12.86 ± 0.04	9.21 ± 0.03	>100	9.09 ± 0.01	23.12 ± 0.03
5h	Trp	Ser	8.04 ± 0.19	8.11± 0.01	>100	29.50 ± 0.06	45.19 ± 0.07
celecoxib			19.3 ± 0.04	16.0 ± 0.03	18.2 ± 0.01	16.0 ± 0.02	>100

[a]: IC_{50}: drug concentration that inhibits cell growth by 50%.

activity against all chosen cell lines. Compound (2g) showed a great anti-cancer activity against MCF-7, HT-29, and A549 cell lines. Consequently, our results showed that the presence of amino acids such as histidine or phenylalanine increased cytotoxicity in comparison with compounds containing tyrosine and tryptophan. The cytotoxicity activity of the compounds on human fibroblasts showed no significant harmful effects. Based on the MTT assay and structure similarity between modified tetrapeptide compounds (1e-5h) and Celecoxib, it could be assumed that one of the mechanisms for cytotoxic activity of these compounds on different cell lines are mediated through COX-2 receptors.

Therefore, the orientation of compound 3g as the most potent compound against MCF-7, in the COX-2 active site was examined by a docking experiment (Fig 2). This molecular modeling study showed that compound 3g was well bound into the active site of COX-2 receptor so that the N atom of the imidazole ring of His^{90} is in the vicinity of the oxygen of sulfonyl group (distance=3.78 A˚) and is capable of binding to this moiety. In addition, docking showed the hydrophobic pocket surrounding the isoleucine side chain by the residues Leu^{531}, and Leu^{359}. In addition, molecular modeling studies



Scheme 1. Reagents and conditions: a) DIC, HOBT, DMAP, Fmoc-Asp, shaking 2 h; b) Piperazine 10% in DMSO, 30 min; c) DIC, HOBT, Fmoc-Phe ,shaking 3 h; d) Piperazine 10% in DMSO, 30 min e) DIC, HOBT, Fmoc-Gly ,shaking 3 h; f) Piperazine 10% in DMSO,30 min; g) DIC, HOBT, 4-(Methylsulfonyl)benzoic acid, shaking 3 h; h) trifluoroacetic acid/DCM/anisole /triisopropyl-silane ,2 h.

(Fig 3) showed the good superimposition of compound 3g with Celecoxib as a crystallography compound in the COX-2 active site. These data together with biological results are in agreement that one of the mechanisms of cytotoxic activity of compounds (1e-5h) on these cell lines might be mediated through acting on COX-2 receptor.

Conclusion

This study indicates that the most of the synthesized compounds showed moderate to good cytotoxicity against different cell lines. In addition, modifications on the basic side chain of amino acids had a significant influence on the cell cytotoxicity.

Acknowledgements

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Competing interests

The authors declare no conflict of interest.

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