Journal of Receptors and Signal Transduction

ISSN: 1079-9893 (Print) 1532-4281 (Online) Journal homepage: https://www.tandfonline.com/loi/irst20

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To cite this article: Mandala Chandrasekhar, Gandavaram Syam Prasad, Chintha Venkataramaiah, Kollu Umapriya, Chamarthi Naga Raju, Kalluru Seshaiah & Wudayagiri Rajendra (2020): *In silico* and *in vitro* antioxidant and anticancer activity profiles of urea and thiourea derivatives of 2,3-dihydro-1*H*-inden-1-amine, Journal of Receptors and Signal Transduction, DOI: 10.1080/10799893.2019.1710848

To link to this article: https://doi.org/10.1080/10799893.2019.1710848

Published online: 07 Jan 2020.

JOURNAL OF RECEPTORS AND SIGNAL TRANSDUCTION
https://doi.org/10.1080/10799893.2019.1710848

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RESEARCH ARTICLE

In silico and *in vitro* antioxidant and anticancer activity profiles of urea and thiourea derivatives of 2,3-dihydro-1*H*-inden-1-amine

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ABSTRACT

Synthesis of a series of new urea and thiourea compounds have been accomplished by the reaction of 2,3-dihydro-1*H*-inden-1-amine with various phenyl isocyanates and isothiocyanates. These compounds were evaluated for their antioxidant activity by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and nitric oxide (NO) radical scavenging assay methods including IC_{50} values. Some of the compounds exhibited potential activity in the two tested methods. Among the series of compounds, urea derivative linked with 4-bromo phenyl ring (**4b**), and thiourea derivatives bonded with phenyl ring (**4e**), 4-fluoro phenyl ring (**4f**) and 4-nitro pheyl ring (**4h**) were found to exhibit promising anti oxidant activity with low IC_{50} values. Where four of the title comounds exhibited higher bindig energies than the reference compound (Imatinib) in *in silico* molecular docking studies with Aromatase. All the synthesized compounds were characterized by IR, ^{1}H, ^{13}C NMR and mass spectral data.

ARTICLE HISTORY
Received 8 November 2019
Revised 20 December 2019
Accepted 23 December 2019

KEYWORDS
Antioxidant activity (DPPH/NO); aromatase; inden-1H-1-amine; molecular docking; thiourea derivatives; urea derivatives

Introduction

Inden is a non-heterocyclic compound and this is a fused ring of benzene ring with penta-cyclic ring. It is well-known that indene chemistry has a very long history due to these derivatives have found in numerous biologically active molecules [1–14]. The inden derivatives having the substitutions on either benzene ring or on five membered ring are valuable synthetic targets in organic and medicinal chemistry because of their important biological activities and applications in functional materials [15]. For example, Resagiline, Braziline and Brx-019 are important inden drug candidates (Figure 1).

Inden scaffolds have found a prompt place in the carbocyclic products because of the presence of these moieties in various natural products and in many drug candidates possessing potential estrogenic bioactivity [16]. Also, these derivatives have been exhibited potential biological activities such as analgesic [17], myorelaxation, immunomodulatory properties [18], type-I diabetic activities [19] and luminescent properties. In addition, bromoindenones are important precursors for synthesis of ninhydrin derivatives and aminoindanes are useful building blocks for many biologically important compounds such as HIV (human immunodeficiency virus) protease inhibitors and neuroprotective agents [20].

Further, urea and thiourea derivatives are the most multipurpose bioactive molecules and have been reported as antibacterial, antifungal, antitubercular, anti-inflammatory, antithyroid, antihelmintic, rodenticidal, insecticidal, herbicidal and plant growth regulatory properties. Benzoylphenyl urea compounds are one class of insect growth regulators, which inhibit chitin synthesis which is responsible for the formation of insect cuticle, *N*-(1,2,4-triazol-3-yl)-*N*′arylthiourea acts as an effective uncoupler of oxidative phosphorylation in mitochondria. Due to the important applications of urea and thiourea derivatives, many research groups have been interested to develop the possibility of next generation urea and thiourea molecules which could be more potent as chemotherapeutic agents.

In continuation our work [21–23] on synthesis of bioactive urea and thiourea derivatives, new urea and thiourea derivatives of 2,3-dihydro-1*H*-inden-1-amine **4a–j** has been synthesized by reacting 2,3-dihydro-1*H*-inden-1-amine (**2**) with various substituted phenyl isocyanates **3a–d** and isothiocyanates **3e–j** and evaluated their antioxidant activity. The synthesized molecules were also studied for evaluation of breast cancer activity molecular docking was done with Aromatase enzyme.

Materials and methods

Chemistry

All the solvents and chemicals were purchased from Merck and Sigma-Aldrich respectively and were used without further purification. Melting points of the compounds were determined in open capillary tube on Guna melting point apparatus and are uncorrected. Infrared spectra were recorded on Bruker ALPHA interferometer instrument. ^{1}H and ^{13}C NMR were recorded on Bruker 400 MHz instrument. TMS was used as a standard in $CDCl_3$ solutions, in the recording of proton and carbon 13 NMR. Chemical shift and coupling

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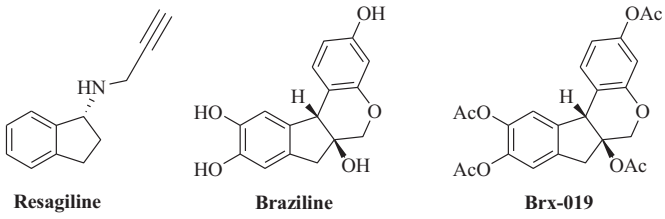


Figure 1. Inden derivatives.

constant were expressed in ppm and Hz respectively. Mass spectra were recorded on MLP 2103 mass spectrometer.

General procedure for the synthesis of urea and thiourea derivatives of 2,3-dihydro-1H-inden-1-amine (4a-j)

A mixture of 2,3-dihydro-1*H*-inden-1-amine hydrochloride (**1**) (250 mg, 1.48 mmol) and triethylamine (Et_3N) (0.25 mL, 1.78 mmol) were taken into a round bottom flask containing THF (10 mL). The mixture was stirred for 3.0 h at 60 °C. The solid, triethylamine hydrochloride ($Et_3N{\cdot}HCl$) salt was removed by filtration as a residue and washed the bed with 3 mL of THF. The combined filtrate containing free base of 2,3-dihydro-1*H*-inden-1-amine (**2**) was transferred into a clean round bottom flask. Triethyl amine (0.24 mL, 1.77 mmol) and 1-isocyanato-4-nitrobenzene (**3c**) (241 mg, 1.47 mmol) were added to the reaction mass. The reaction mixture was stirred at 45–50 °C for 2 h. After completion of the reaction as checked by TLC, the reaction mixture was concentrated under vacuum to obtain crude 1-(2,3-dihydro-1*H*-inden-1-yl)-3-(4-nitrophenyl)urea (**4c**). The pure desired product, **4c** was isolated by column chromatography using 10–30% ethyl acetate: *n*-hexane as an eluent. The same procedure was adopted for the synthesis of the remaining title compounds.

1-(2,3-Dihydro-1H-inden-1-yl)-3-(4-fluorophenyl)urea (4a).

White solid, Yield (%): 73; mp: 210–212 °C; IR (cm^{-1}): 3298 (N–H), 3152 (N–H), 1629 (C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 9.62 (1H, s, NH), 8.66 (1H, s, NH), 7.45–7.38 (2H, m, H_{Ar}), 7.28–7.16 (3 H, m, H_{Ar}), 7.12–7.02 (2H, m, H_{Ar}), 6.46 (1H, d, $J = 8.0$ Hz, H_{Ar}), 5.18 (1H, dd, $J = 7.6$, 8.0 Hz, CH–NH), 2.95–2.88 (1H, m, CH_2), 2.83–2.75 (1H, m, CH_2), 2.45–2.39 (1H, m, CH_2), 1.80–1.70 (1H, m, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.2 (d, $J = 189.5$ Hz), 155.1, 144.4, 142.8, 136.7, 127.4, 126.4, 124.6, 123.8, 119.2, 115.3, 54.4, 33.9, 29.6; ESI-MS (*m/z*): 271 $[M + H]^+$, 249, 155.

1-(4-Bromophenyl)-3-(2,3-dihydro-1H-inden-1-yl)urea (4b).

White solid, Yield (%): 82; mp: 304–306 °C; IR (cm^{-1}): 3288 (N–H), 3198 (N–H), 1630 (C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 9.72 (1H, s, NH), 8.54 (1H, s, NH), 7.72–7.67 (2H, m, H_{Ar}), 7.60–7.55 (2H, m, H_{Ar}), 7.46–7.37 (3 H, m, H_{Ar}), 6.49 (1H, d, $J = 8.0$ Hz, H_{Ar}), 5.28 (1H, d, $J = 8.0$ Hz, CH–NH), 2.95–2.86 (1H, m, CH_2), 2.82–2.76 (1H, m, CH_2), 2.46–2.40 (1H, m, CH_2), 1.84–1.76 (1H, m, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 154.5, 144.3, 142.8, 137.4, 132.1, 127.3, 126.4, 124.5, 123.8, 122.3, 121.7, 54.4, 33.8, 29.6; ESI-MS (*m/z*): 330 $[M]^+$, 332 $[M + 2]^+$, 198.

1-(2,3-Dihydro-1H-inden-1-yl)-3-(4-nitrophenyl)urea (4c).

Greenish yellow solid, Yield (%): 88; mp: 338–340 °C; IR (cm^{-1}): 3356(N–H), 3227 (N–H), 1583 (C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 9.72 (1H, s, NH), 8.54 (1H, s, NH), 7.72–7.67 (2H, m, H_{Ar}), 7.60–7.55 (2H, m, H_{Ar}), 7.46–7.37 (3 H, m, H_{Ar}), 6.49 (1H, d, $J = 8.0$ Hz, H_{Ar}), 5.28 (1H, d, $J = 8.0$ Hz, CH–NH), 2.95–2.86 (1H, m, CH_2), 2.82–2.76 (1H, m, CH_2), 2.46–2.40 (1H, m, CH_2), 1.84–1.76 (1H, m, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.9, 146.2, 144.4, 143.9, 142.6, 127.2, 126.4, 124.5, 123.8, 122.9, 120.4, 54.5, 33.9, 29.6; ESI-MS (*m/z*): 298 $[M + H]^+$, 182, 117.

1-(3,4-Dichlorophenyl)-3-(2,3-dihydro-1H-inden-1-yl)urea (4d).

White solid, Yield (%): 82; mp: 198–200 °C; IR (cm^{-1}): 3298 (N–H), 3109 (N–H), 1589 (C=O); ^1H NMR (400 MHz, DMSO-d_6): δ 9.13 (1H, s, NH), 8.69 (1H, s, NH), 7.87 (1H, dd, $J = 2.4$, 5.2Hz, H_{Ar}), 7.52 (1H, d, $J = 8.8$ Hz, H_{Ar}), 7.46 (1H, d, $J = 8.8$ Hz, H_{Ar}), 7.35 (1H, dd, $J = 2.4$, 6.4 Hz, H_{Ar}), 7.28–7.16 (2H, m, H_{Ar}), 6.66 (1H, d, $J = 8.0$ Hz, H_{Ar}), 5.18 (1H, dd, $J = 7.6$, 8.0 Hz, CH–NH), 2.93–2.88 (1H, m, CH_2), 2.83–2.75 (1H, m, CH_2), 2.47–2.39 (1H, m, CH_2), 1.82–1.73 (1H, m, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 154.7, 144.1, 142.8, 139.5, 131.0, 130.6, 130.4, 127.5, 126.4, 124.6, 123.8, 122.5, 121.0, 54.4, 33.6, 29.6; ESI-MS (*m/z*): 321 $[M + H]^+$, 323 $[M + H + 2]^+$, 325 $[M + H + 4]^+$.

1-(2,3-Dihydro-1H-inden-1-yl)-3-phenylthiourea (4e).

Brown solid, Yield (%): 76; mp: 118–120 °C; IR (cm^{-1}): 3363 (N–H), 3151 (N–H), 1373 (C=S); ^1H NMR (400 MHz, DMSO-d_6): δ 9.43 (1H, s, NH), 8.07 (1H, d, $J = 8.0$ Hz, H_{Ar}), 7.50–7.44 (2H, m, H_{Ar}), 7.36–7.33 (1H, m, H_{Ar}), 7.26–7.17 (3 H, m, H_{Ar}), 7.16–7.10 (2H, m, H_{Ar}), 5.88 (1H, d, $J = 7.2$Hz, CH–NH), 2.95–2.89 (1H, m, CH_2), 2.85–2.77 (1H, m, CH_2), 2.53–2.45 (1H, m, CH_2), 1.92–1.82 (1H, m, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.9, 144.3, 142.8, 136.7, 130.3, 127.6, 126.4, 126.2, 124.6, 124.0, 123.8, 58.6, 33.9, 29.6. ESI-MS (*m/z*): 269 $[M + H]^+$, 153, 102.

1-(2,3-Dihydro-1H-inden-1-yl)-3-(4-fluorophenyl)thiourea (4f).

White solid, Yield (%): 73; mp: 164–166 °C; IR (cm^{-1}): 3297 (N–H), 1388 (C=S); ^1H NMR (400 MHz, DMSO-d_6): δ 9.46 (1H, s, NH), 8.06 (1H, d, $J = 8.0$ Hz, H_{Ar}), 7.55–7.46 (2H, m, H_{Ar}), 7.38–7.30 (2H, m, H_{Ar}), 7.27–7.19 (2H, m, H_{Ar}), 7.15–7.08 (2H, m, H_{Ar}), 5.86 (1H, dd, $J = 7.2$, 7.6 Hz, CH–NH), 3.02–2.95 (1H, m, CH_2), 2.90–2.84 (1H, m, CH_2), 2.52–2.44 (1H, m, CH_2), 1.89–1.80 (1H, m, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.8, 158.9 (d, $J = 191.8$ Hz), 143.4, 143.9, 142.9, 135.7, 127.6, 126.3, 125.5, 124.6, 124.0, 115.1, 58.8, 32.7, 29.6; ESI-MS (*m/z*): 287 $[M + H]^+$.

1-(3-Chlorophenyl)-3-(2,3-dihydro-1H-inden-1-yl)thiourea (4g).

White solid, Yield (%): 85; mp: 245–247 °C; IR (cm^{-1}): 3255 (N–H), 1334 (C=S); ^1H NMR (400 MHz, DMSO-d_6): δ 9.46 (1H, s, NH), 8.06 (1H, d, $J = 8.0$ Hz, H_{Ar}), 7.55–7.46 (2H, m, H_{Ar}), 7.38–7.30 (2H, m, H_{Ar}), 7.27–7.19 (2H, m, H_{Ar}), 7.15–7.08 (2H, m, H_{Ar}), 5.88 (1H, dd, $J = 7.2$, 7.6 Hz, CH–NH), 3.02–2.95 (1H, m, CH_2), 2.90–2.84 (1H, m, CH_2), 2.52–2.44 (1H, m, CH_2), 1.89–1.80 (1H, m, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ

180.6, 144.4, 142.8, 139.8, 135.4, 131.2, 129.4, 127.5, 126.4, 125.9, 124.4, 123.7, 58.6, 33.8, 29.6; ESI-MS (*m/z*): 303 $[M+H]^+$, 305 $[M+H+2]^+$.

1-(2,3-Dihydro-1H-inden-1-yl)-3-(4-nitrophenyl)thiourea (4h). White solid, Yield (%): 89; mp: 190-192 °C; IR (cm^{-1}): 3300 (N–H), 3192 (N–H), 1329 (C=S); ^1H NMR (400 MHz, DMSO-d_6): δ 9.36 (1H, s, NH), 8.12 (1H, d, $J=8.0$ Hz, H_{Ar}), 8.08–8.02 (1H, m, H_{Ar}), 7.45–7.34 (3 H, m, H_{Ar}, NH), 7.25–7.18 (2H, m, H_{Ar}), 7.14–7.08 (2H, m, H_{Ar}), 5.86 (1H, dd, $J=7.2$, 7.6 Hz, CH–NH), 3.00–2.86 (2H, m, CH_2), 2.52–2.48 (1H, m, CH_2), 1.91–1.85 (1H, m, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.2, 144.9, 144.3, 143.4, 142.8, 127.4, 126.5, 125.1, 124.5, 124.1, 123.7, 58.7, 33.8, 29.6; ESI-MS (*m/z*): 314 $[M+H]^+$.

1-(3-Bromophenyl)-3-(2,3-dihydro-1H-inden-1-yl)thiourea (4i). Pale brown solid, Yield (%): 80; mp: 140–142 °C; IR (cm^{-1}): 3303 (N–H), 1348 (C=S); ^1H NMR (400 MHz, DMSO-d_6): δ 9.66 (1H, s, NH), 8.32 (1H, d, $J=8.0$ Hz, H_{Ar}), 7.94 (1H, s, H_{Ar}), 7.42–7.18 (7 H, m, H_{Ar}, NH), 5.87 (1H, d, $J=7.6$ Hz, CH–NH), 3.08–2.78 (2H, m, CH_2), 2.54–2.51 (1H, m, CH_2), 1.92–1.83 (1H, m, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.3, 144.4, 142.8, 138.4, 129.6, 128.0, 127.4, 126.5, 126.0, 124.5, 124.1, 123.8, 122.8, 58.8, 33.9, 29.6; ESI-MS (*m/z*): 347 $[M+H]^+$, 349 $[M+H+2]^+$.

1-(2,4-Difluorophenyl)-3-(2,3-dihydro-1H-inden-1-yl)thiourea (4j). White solid, Yield (%): 80; mp: 242–244 °C; IR (cm^{-1}): 3274 (N–H), 3191 (N–H), 1321 (C=S); ^1H NMR (400 MHz, DMSO-d_6): δ 9.35 (1H, s, NH), 8.22 (1H, d, $J=8.0$ Hz, H_{Ar}), 7.45–7.28 (5 H, m, H_{Ar}), 7.25–7.20 (2H, m, H_{Ar}), 7.17 (1H, s, H_{Ar}), 5.88 (1H, d, $J=7.2$, 7.6 Hz, CH–NH), 3.05–2.80 (2H, m, CH_2), 2.49–2.45 (1H, m, CH_2), 1.93–1.88 (1H, m, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 179.8, 165.4 (d, $J=239.5$ Hz), 159.2 (d, $J=180.2$Hz), 144.3, 142.8, 130.2, 127.5, 126.4, 124.4, 124.1, 123.7, 116.4, 112.1 (d, $J=47$ Hz), 105.4, 58.7, 33.9, 29.6; ESI-MS (*m/z*): 289 $[M+H]^+$.

Molecular docking studies

The three-dimensional structure of Aromatase and Imatinib (Figure 2) were downloaded from the RCSB protein Data Bank. The atomic coordinates of the protein was estranged and geometry optimization was done using Argus Lab 4.0.1 [24].

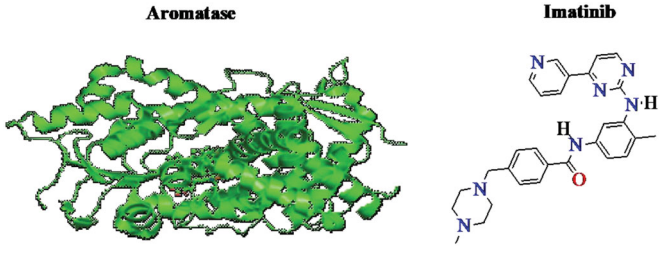


Figure 2. Three-dimensional structure of Aromatase and Imatinib.

Ligand selection

The chemical structures of compounds were prepared using ChemBioDraw and all the ligands were converted into Pdbqt file format and atomic coordinates were generated using Pyrx 2010.12

Analysis of target active binding sites

The active sites are the coordinates of the ligand in the original target protein grids, and these active binding sites of target protein were analyzed using the Drug Discovery Studio version 3.0 and 3D Ligand Site virtual tools [25].

Molecular docking studies were carried against Aromatase protein with compounds **4a–j**, the reference drug Imatinib, using the docking module implemented in Pyrx 2010.12. Initially the protein structures were protonated with the addition of polar hydrogens, followed by energy minimization with the MMFF94x force field, in order to get the stable conformer of the proteins. Flexible docking was employed, the inhibitor binding site residues were softened and highlighted through the 'Site Finder' module implemented in the Pymol software [26]. The grid dimensions were predicted as X: 28.27, Y: 27.13, Z: 28.51 for Aromatase respectively. The docking was carried out with the default parameters i.e. placement: triangle matcher, recording 1: London dG, refinement: force field and a maximum of 10 conformations of each compound were allowed to be saved in a separate database file in a .mdb format. After the docking process, the binding energy and binding affinity of the protein-ligand complexes were calculated using Pymol viewer tool (www.pymol.org).

Structural analysis and visualization

Protein and ligand interactions were analyzed and visualized through Pymol viewer tool (www.pymol.org).

In vitro *anti cancer activity*

The Human Breast Cancer cell line, MCF-7 was procured from King Institute of PreventiveMedicine, Chennai. The cells were grown in culture flask using Minimum Essential Medium supplemented with 3% *L*-Glutamine, 10% Fetal bovine serum, Penicillin (100 IU/mL), Streptomycin (100 μg/mL) and Amphotericin B along with 7.5% sodium bicarbonate in a T 25 mL cultured vented flask and incubated at 37 °C in 5% CO_2 incubator. After 3 days, about 80–90% confluent monolayer (adherent) formation was confirmed by inverted microscope. Then it was sub cultured by using TPVG solution along with minimum essential medium and used for further study.

Growth inhibition of MCF-7 cells by title compounds were determined by MTT assay. The cells were harvested and seeded in a 96 well plates and the plates were incubated for 24 h at 37 °C in 5% CO_2 for attachment of cells. After 12 h different concentrations of title compounds such as 10 μg/mL, 20 μg/mL, 30 μg/mL were added to the cells and incubated for 24 h. After incubation, the medium was replaced with phenol red and FBS free medium and 15 μL of MTT (5 mg/mL) dye was added per well and wrapped with

aluminum foil and the plate was incubated again for 4 h. After incubation medium was aspirated and 100 µL of DMSO were added to each well to solubilize the formazan crystals [27]. The optical density (OD) was measured at the wavelength of 570 nm. The percentage of cell inhibition was determined by following formula:

$$\%\ \text{of cell viability} = \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

Antioxidant activity

DPPH radical scavenging assay

The DPPH radical scavenging method [28] was used to evaluate the antioxidant property. The antioxidant activity was compared with that of the natural antioxidant, ascorbic acid. The concentrations of the synthetic compounds required to scavenge DPPH showed a dose dependent response. The antioxidant activity of each sample was expressed in terms of IC_{50}, and was calculated from the graph after plotting inhibition percentage against compound concentration. 1.0 mL of various concentrations of the compound (25, 50, 75 and 100 µg/mL) in methanol was added to 4 mL of 0.004% (w/v) methanol solution of DPPH. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a spectrophotometer. The solution without any compound and with DPPH and methanol was used as control. The experiment was replicated in three independent assays. Ascorbic acid was used as positive control. Inhibition of DPPH free radical in percentage was calculated by the formula:

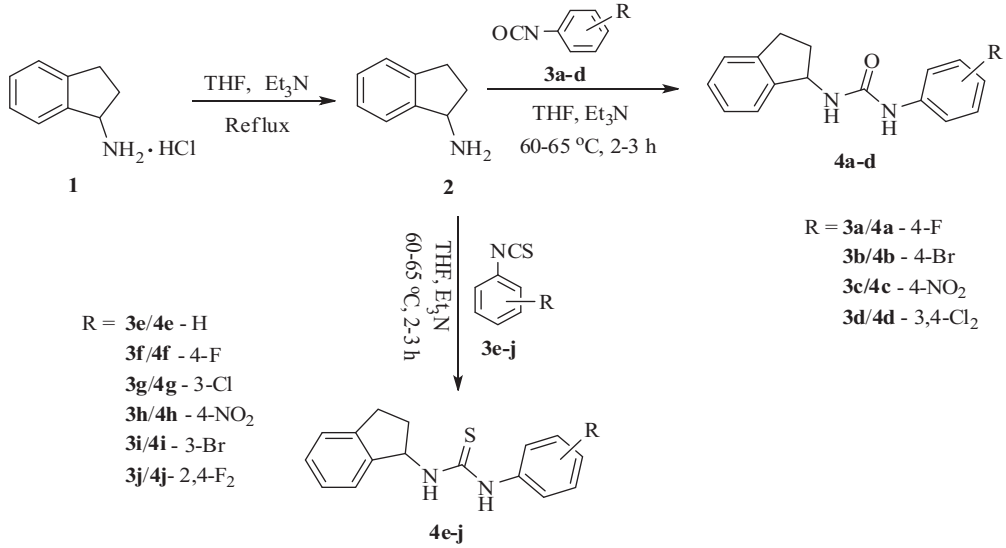
$$\%\,\text{Inhibition} = \frac{(A_{\text{control}} - A_{\text{test}})}{A_{\text{control}}} \times 100$$

where $A_{control}$ is the absorbance of the control (*L*- Ascorbic acid) and A_{test} is the absorbance of reaction mixture samples (in the presence of compounds). All tests were run in triplicates ($n = 3$), and average values were calculated. Inhibition concentration (IC_{50}) parameter was used for the interpretation of the results from DPPH method. The discoloration of the sample was plotted against the sample concentration in order to calculate the IC_{50} value. It is defined as the amount of the sample necessary to decrease the absorbance of DPPH by 50%.

NO radical scavenging assay

NO radical scavenging assay can be estimated by the use of Griess Illosvoy reaction [29]. The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO^{-}. Under aerobic conditions, NO^{-} reacts with oxygen to produce stable products (nitrate and nitrite). The quantities can be determined using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. 1.0 mL of various concentrations of the compound (25, 50, 75 and 100 µg/mL) in methanol was added to 4 mL of 0.004% (w/v) methanol solution. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. After the incubation period, 0.5 mL of Griess reagent was added. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride was immediately read at 550 nm. Inhibition of nitrite formation by the plant extracts and the standard antioxidant ascorbic acid were calculated relative to the control. Inhibition data (percentage inhibition) were linearized against the concentrations of each extract and standard antioxidant. IC_{50} which is an inhibitory concentration of each extract required to reduce 50% of the nitric oxide formation was determined.

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$



Scheme 1. Synthesis of urea and thiourea derivatives of 2,3-dihydro-1*H*-inden-1-amine **4a–j**.

Table 1. *In vitro* anticancer activity of the compounds against MCF-7 cell lines.

	% of Inhibition			
Compound	10 μg/mL	20 μg/mL	30 μg/mL	IC_{50} (μg)
4a	58.24 ± 1.28	68.36 ± 2.32	77.24 ± 3.32	5.24 ± 0.12
4b	33.25 ± 3.22	46.28 ± 4.12	69.32 ± 1.28	21.46 ± 0.22
4c	52.12 ± 1.22	66.24 ± 2.22	78.26 ± 3.12	6.12 ± 0.16
4d	48.48 ± 2.12	60.22 ± 1.14	68.22 ± 1.14	17.46 ± 0.72
4e	26.28 ± 4.23	48.88 ± 2.38	60.12 ± 4.22	21.22 ± 0.46
4f	30.66 ± 2.46	54.86 ± 1.86	58.24 ± 1.12	19.17 ± 0.40
4g	30.12 ± 1.16	50.16 ± 2.46	61.84 ± 2.84	19.66 ± 0.60
4h	48.56 ± 1.86	61.22 ± 4.24	68.12 ± 1.36	11.86 ± 0.16
4i	26.48 ± 2.66	49.18 ± 3.24	62.54 ± 1.28	23.18 ± 0.42
4j	32.28 ± 3.26	51.22 ± 2.86	59.36 ± 1.68	19.66 ± 0.46
2	20.88 ± 3.22	38.12 ± 1.96	51.28 ± 1.48	28.66 ± 0.66
Control	100	100	100	–

Results and discussion

Chemistry

Initially, 2,3-dihydro-1*H*-inden-1-amine hydrochloride (**1**) was made as a free base on treating with Et_3N to obtain 2,3-dihydro-1*H*-inden-1-amine (**2**) and used without further purification to the next process. 2,3-Dihydro-1*H*-inden-1-amine (**2**) was treated with various substituted phenyl isocyanates **3a–d** and isothiocyanates **3e–j** in the presence of Et_3N under heating conditions (10–40 °C) to afford substituted 1-(2,3-dihydro-1*H*-inden-1-yl)-3-phenyl urea derivatives **4a–d** and 1-(2,3-dihydro-1H-inden-1-yl)-3-phenylthiourea derivatives **4e–j** in moderate yields (Scheme 1). The structures of all the

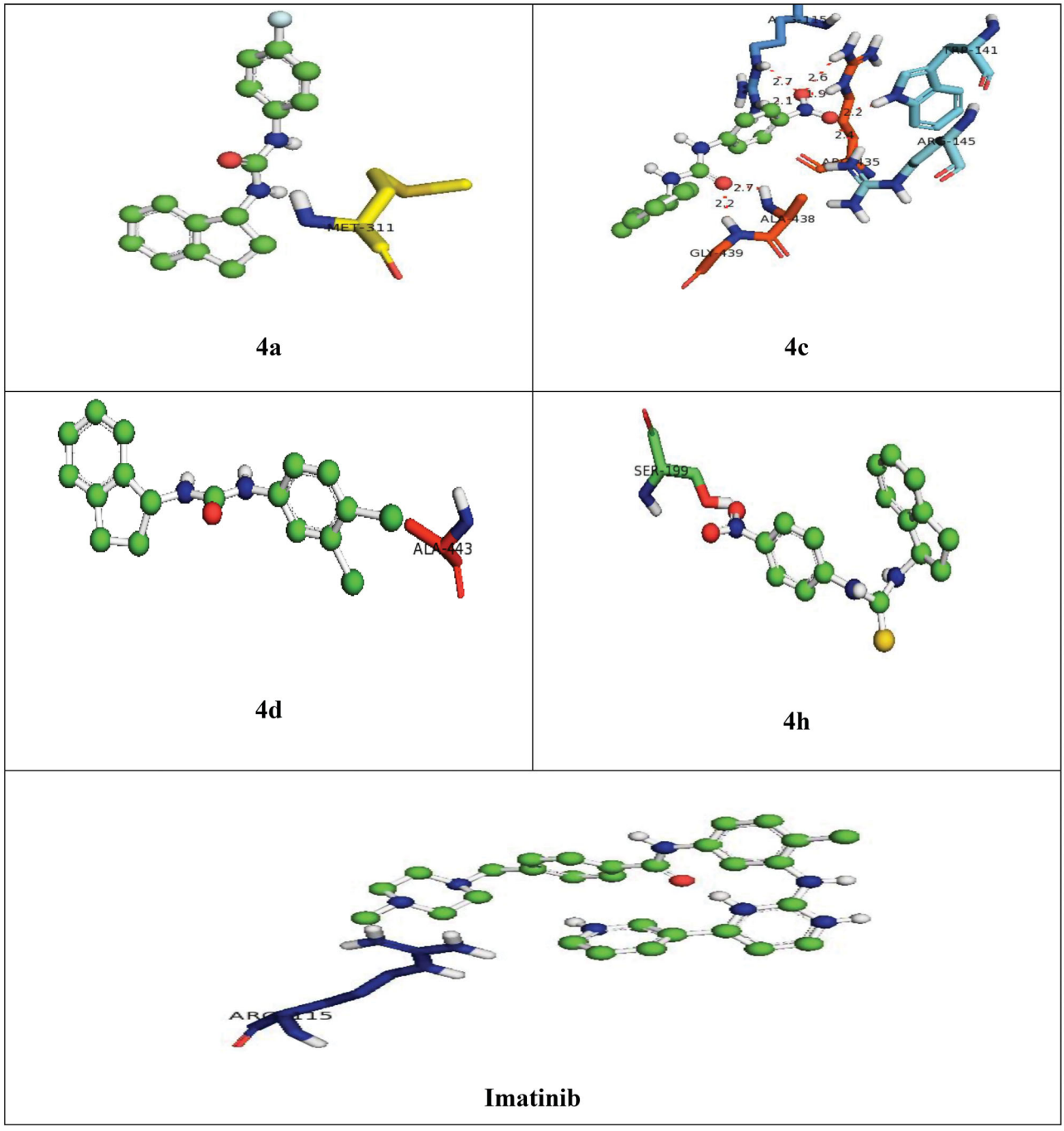


Figure 3. Diagrammatic representation of 3D modeled binding modes of the compounds **4a–j** and standard with the binding domain of Aromatase.

Table 2. Bonding characterization of synthesized compounds **4a–j** and Imatinib (Reference drug) against human placental aromatase protein.

Compound	Binding energy (kcal/mol)	Binding interaction	Bond Length (Å)	Bond Angle (°)	Bond Type
Imatinib	−9.2	Arg 115 CB … HN	2.2	109.9	H-don
4a	−9.3	Met 311 CZ … .HN	2.7	121.8	H-don
4b	−7.9	Arg 115 CB … .HC	2.4	108.7	H-acc
4c	−10.2	Met 149 CA … .OC	2.7	83.7	H-acc
		Gly 439 CZ … .OC	2.2	104.8	H-acc
		Arg 145 CZ … ..ON	2.4	121.6	H-acc
		Trp 141 CB … .ON	2.2.	136.0	H-acc
		Arg 435 CZ … .ON	2.6	107.3	H-acc
		Arg 435 CZ … .ON	1.9	121.3	H-acc
		Arg 115 CB … .ON	2.7	106.3	H-acc
		Arg 115 CB … .ON	2.1	89.7	H-acc
4d	−9.8	Ala 443 CA … ..ON	2.8	106.7	H-acc
4e	−7.5	Arg 115 CZ … .ON	2.1	101.8	H-acc
4f	−7.6	Ser 478CZ … ..ON	2.4	89.9	H-acc
4g	−7.6	Ile 133 CB … ..HN	2.1	104.7	H-don
4h	−9.5	Ser 199 CZ … ..HN	2.8	76.8	H-don
4i	−7.6	Ile 133 CB … .HN	2.9	78.9	H-don
4j	−7.8	Asp 309 CA … .ON	1.9	123.3	H-acc

Table 3. DPPH free radical scavenging activity of the synthesized compounds **4a–j**.

Compound	25 μg/mL	50 μg/mL	75 μg/mL	100 μg/mL
4a	20.22	56.82	60.79	87.70
4b	31.30	40.14	51.62	63.71
4c	16.76	22.35	51.09	61.80
4d	9.24	16.56	49.81	68.45
4e	35.77	47.76	55.38	67.78
4f	33.84	48.88	57.31	62.90
4g	22.56	36.81	52.17	60.54
4h	29.06	40.85	50.81	65.65
4i	23.88	38.71	46.74	60.16
4j	26.21	51.97	63.13	78.41
2	11.07	45.54	62.35	79.67
Std	46.54	66.66	73.78	79.47

Std: Ascorbic Acid.

Table 4. Nitric oxide scavenging activity of the title compounds **4a–j**.

Compound	25 μg/mL	50 μg/mL	75 μg/mL	100 μg/mL
4a	24.95	47.77	66.81	82.12
4b	27.36	37.72	55.02	70.08
4c	25.62	30.84	57.47	68.83
4d	26.28	34.07	51.45	63.44
4e	29.93	41.62	51.57	61.60
4f	30.43	37.39	47.59	57.37
4g	28.60	38.05	50	56.71
4h	29.02	38.39	50.91	57.04
4i	27.94	44.82	62.62	72.23
4j	28.02	36.81	45.60	50.41
2	25.37	28.44	47.81	68.75
Std	38.14	47.01	57.96	67.74

Std: Ascorbic Acid.

newly synthesized compounds were characterized by IR, NMR (^{1}H and ^{13}C) and mass spectral data. The compounds **4a–j** showed characteristic IR stretching absorptions for N–H, C=O and C=S in the region [30] 3363–3248, 1630–1583 and 1388–1321 cm^{-1} respectively. The aromatic protons of benzene rings of **4a–j** showed signals in the region δ 8.69–6.49. The protons of N–H resonated at δ 9.72–9.13. The C-H proton attached to nitrogen showed a signal as doublet (d)/doblet of doublet (dd) in the region δ 5.88–5.18, due to coupling with proton(s) of neighboring carbon in five membered ring of inden moiety. The aliphatic protons of $\underline{CH_2}$-$\underline{CH_2}$ showed signal(s) as multiplet in the region δ 3.08–1.70. The aromatic carbons resonated in the region δ 158.2–105.4 based on the functionalities attached to phenyl ring for all the compounds **4a–j**. The carbon chemical shifts for carbonyl group of **4a–d** and thiocarbonyl group of **4e–j** appeared in the region δ 155.1–153.9 and 180.9–179.8 respectively.

Molecular docking studies

In order to provide strength to the synthesized compounds, docking analysis was carried out for compounds **4a–j** with selective pharmacological targets such as Aromatase protein of breast cancer which is involved in the pathogenesis and induction of cancer. The crystal structure of aromatase (PDB id: 3S7S) was retrieved from the protein data bank. The docking results of ligands against Aromatase showed that the compounds **4a–j** have significant binding modes, with dock scores of –9.3, –9.9, –10.2, –9.8, –7.5, –7.6, –7.6, –9.5, –7.6 and –7.8 respectively, among all four compounds were shown higher dock scores when compared with the control drug Imatinib (–9.2) except **4b**, **4e**, **4f**, **4g**, **4i** and **4j**. The H-bonds, binding affinities and energy profiles of compounds **4a–j** toward the active site amino acids of the enzyme are summarized in Table 1 and their 3D modeled interactions of the title compounds with aromatase protein were shown in Figure 3. Thus these interactions provide support for the significant decrease in Aromatase activity. Hence, the present investigation demonstrate that the synthesized compounds will be the promising next generation chemotherapeutic drugs, which can be effectively used in the treatment of breast cancer and other related disorders. Compounds except **4c**, all the title compounds have showed hydrophobic interactions against 3S7S protein.

In vitro *anti cancer activity*

The results obtained from this study, it is observed that all the title compounds have shown promising anti cancer activity on MCF-7 cell lines (Table 2). All the title compounds inhibited the cell viability significantly on dose dependent manner. The maximum of 78% of viability reduction was observed in the compound, **4c** treated with the

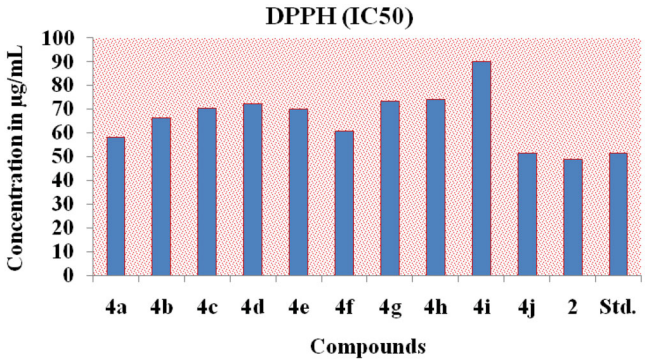


Figure 4. Half inhibitory concentration of compounds by DPPH method.

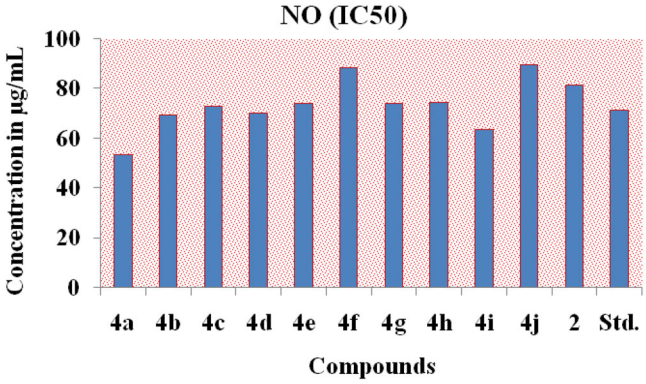


Figure 5. Half inhibitory concentration of title compounds by NO method.

concentration of 30 µg/mL. Whereas the majority of the compounds exhibited potent anti cancer activity on MCF-7 cell lines. Amongst the compounds **4a**, **4c**, **4d** and **4h** have shown prominent anti cancer activity 77.24%, 78.26%, 68.22% and 68.12% and the remaining compounds also exhibited good anti cancer activity than that of the compound **2**.

Antioxidant activity

The newly synthesized urea and thiourea compounds **8a–j** were screened for their antioxidant activity. DPPH radical scavenging assay and NO radical scavenging assay were used to examine the antioxidant nature of compounds. The antioxidant nature of newly synthesized compounds were investigated at different concentrations such as 25, 50, 75 and 100 µg/mL, (Tables 3 and 4) and ascorbic acid was used as a standard to compare the activity. As well as, half maximal concentration (IC_{50}) of the compounds was tested using DPPH and NO method (Figures 4 and 5). The experimental results disclosed that all the compounds exhibited moderate to good antioxidant activity. Some of the compounds, urea derivative **4b** and thiourea derivatives **4e**, **4f** and **4h** exhibited potential activity, in conclusion the two tested methods and IC_{50} values were evaluated in both the methods.

Conclusion

A series of new urea and thiourea derivatives of 3-dihydro-1*H*-inden-1-amine were synthesized. The antioxidant activity nature of the title compounds was investigated using DPPH and NO methods including IC_{50} values. Some of the compounds, urea derivative linked with 4-bromo phenyl ring **4b**, and thiourea derivatives bonded with phenyl ring **4e**, 4-fluoro phenyl ring **4f**, and 4-nitro pheyl ring **4h** exhibited potential activity in all the tested methods. The overall screening revealed that thiourea derivatives are acted as antioxidants promisingly as compared with urea derivatives. As an addition molecular docking studies of the synthesized compounds against Aromatase enzyme revealed that majority of the compounds showed higher binding affinities and many more interactions when compared with reference drug, Imatinib. These results suggested that a few of the synthesized compounds can be considered as promising therapeutic candidates for further optimization and development of potential anti-cancer drugs in near future.

Disclosure statement

All the authors declare that no conflict of interest in this work.

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