# SYNTHESIS, SPECTRAL CHARACTERIZATION, MOLECULAR DOCKING STUDIES, ANTI-INFLAMMATORY AND ANTI-BREAST CANCER EVALUATIONS OF COVALENT ORGANIC FRAMEWORK OF DIFFERENTLY SUBSTITUTED ALKYL PHENYLACETATES

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**ABSTRACT:**
Bio-Inhibitors like covalent organic framework of differently substituted alkyl phenylacetates (**DSPA: 2.1-2.12)** to control microbial toxicities and other budding threats were synthesized. The structures of the synthesized drug candidates were characterized through IR, $^{1}$H-NMR, $^{13}$C-NMR spectral data and the molecular weight determination with fragmental futures using Mass Spectrometry. After proper validation of the docking protocol, the *in-silico* analysis of different disease condition like anti-analgesic, anti-inflammatory and anti-cancer targets (PDB ID: **5du1**, **5glw** and **6fe2**) against the synthesized drug candidates **DSPA: 2.1-2.12** have been investigated by AutoDock 4.2 application. The studied docking data analysis revealed that the drug candidates 2-Butyl 4-methoxyphenylacetate (**2.3**) and Methyl 3,4-dimethoxyphenylacetate **(2.5)** are potentially inhibits anti-analgesic/anti-inflammatory proteins **5du1/5glw** and anti-breast cancer protein **6fe2** respectively are useful compounds for the prevention of the diseases. The % of inhibitions at a concentration of 400 µg/ml are found to be 76.6565, 78.8991, 74.5158, 79.8165 and 82.2630 for **2.4a, 2.11a, 2.11, 2.12a** and **diclofenac sodium** respectively, with a biological anti-inflammatory activity order of **2.11< 2.4a < 2.11a < 2.12a < diclofenac sodium**. The synthesized ligands have shown a very impressive anti-breast cancer activity, drug candidate **2.4** with para-methoxy group in its structure has displayed devastating anti-inflammatory effect when compared with the standard **doxorubicin.**

**KEYWORDS:** alkyl phenylacetate, AutoDock 4.2**,** anti-analgesic, anti-inflammatory and anti-cancer.

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## INTRODUCTION:

A well-developed approach in drug advancement on sour taste masking of carboxylic acids[1-3] in oral pharmaceuticals is esterification, and ester linkages of these prodrugs are usually hydrolysed in human body by esterase enzyme present in plasma and various tissues. Due to the presence of dipolar carbonyl and ether linkages in esters act as hydrogen-bond acceptors leads to hydrogen bonding with water and other protic solvents is results a higher water solubility of esters than its equivalent hydrocarbons. The solubility phenomenon of esters is adjusted by different substitution in order to bring the forecasted partition coefficient value and hence the logP, helping to priorities the synthesis of ester analogue for the accountability of a new drug candidate. The prodrug approach has shown many successes[4] and still remains a viable and effective approach to deliver new active agents. This is in fact supported by the recent approved prodrugs and approximately 10% of all marketed drugs worldwide can be considered prodrugs. Since 2008, at least 30 prodrugs have been approved by the FDA,[5] including seven prodrugs in 2015 and six in 2017.

A prominent literature survey revealed that numerous pharmaceutical drugs have been synthesized from phenylacetic acid and used to treat diverse diseases. Cyclopentolate (**Fig. 1**) is a prominent analogue of phenylacetate majestically appear in the World Health Organization's List of Essential Medicines (WHO-LEM)[6] principally used in medications called mydriatics for the purpose to dilate/enlarge the pupil previously eye examinations such as cycloplegic refraction or ophthalmoscopy. Cycloplegic agents prevent the action of acetylcholine at muscarinic receptor sites and based on their mechanism of action, cycloplegics are referred to as anti-cholinergics, anti-muscarinics or parasympatholytic agents.[7-8]

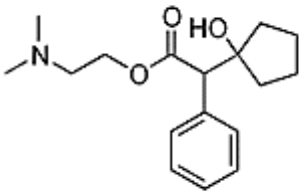


**Fig. 1**

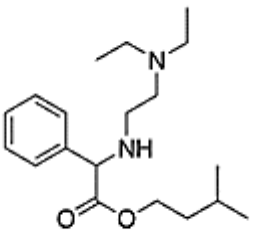


**Fig. 2**

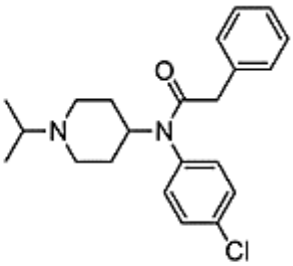


**Fig. 3**

Camylofin (**Fig. 2**) an α-amino analogue of phenylacetate is a smooth muscle relaxant with anticholinergic activity, always being used as a mixture with paracetamol to pleasure pyrexia in addition to stomach ache and this mixture is well known as anticholinergic agent used to block cholinergic receptors or acetylcholine receptors.[9-10] Lorcainide (**Fig. 3**) hydrochloride an antiarrhythmic agent synthesized from phenylacetic acid is restores normal heart rhythm and conduction in patients with premature ventricular contractions, ventricular tachycardiac[11] and Wolff–Parkinson–White syndrome.[12] Phenacemide, Phenindione and Bendazol belongs to phenylacetamide analogues are also synthesized as the derivatives of phenylacetic acid. Hence, the prodrug concepts and derivatization of phenylacetic acid getting importance in the medicinal chemistry.

Since the number of hydrogen bond donors in a molecule is found to enhance the bio-transformational activities[13] on target proteins, successions of a series of hybrid molecules like /

mono-, di-methoxy and tri- methoxy analogs have been synthesized for studies in order to compare with the unsubstituted one. Although trimethoxyphenyl based anticancer agents have recently gained great successes, however none of these natural-derived compounds have been approved by FDA to be marketed as anticancer drug. Since, inconsequential drawbacks including metabolic instability, low oral bioavailability, poor aqueous solubility and undesired side effects have hindered the development of this type of compounds[14-15], further development of such analogue with the efficient therapeutic values is still a continues establishment.

Esterification is a common chemical reaction in which two reactants, alcohol and acid, form an ester as the final reaction product.[16] Esterification can be achieved by many different synthetic pathways; some of these synthetic routes include Fischer

esterification and alcoholysis of acyl chlorides with acid anhydrides. Stegliche esterification is another way of esterification that involves dicyclohexylcarbodimide (DCC) as a coupling reagent using 4-dimethylaminopyridine (DMAP) as a catalyst. The reaction first described by Wolfgang Steglich in 1978 was generally takes place at room temperature with suitable solvent like dichloromethane.[17-18] Herewith, this is proposed to report the synthesis and characterisation of differently substituted phenylacetates.

Traditional Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) such as aspirin, ibuprofen, naproxen and diclofenac are the extensively prescribed small molecule therapeutics to treat fever, inflammation and pain, through inhibiting enzymes cyclooxygenase-1 (COX-1) and -2 (COX-2)[19-22]. Though NSAIDs shows noteworthy lateral gastrointestinal effects, afresh intensifying evidence proposes that it does not exert cardiovascular side effects even administered in a higher dose that provide sustained inhibition of platelet COX-1 throughout the dosing interval like 500 mg twice daily (3– 5). This cardiovascular property has been occupied on cumulative status because the data evolved suggest that the said toxicity first exhibited by rofecoxib and celecoxib extends to other selective or non-selective inhibitors, including diclofenac, indomethacin, and ibuprofen (4, 6, 7). No crystal structures have been reported for naproxen bound to COX-1 or COX-2, and the few naproxen analogs that have been described in the literature have only been tested in vivo (8). However, drug and prodrug investigations to target a variety of enzymes by cluster of esters and its acids confidently shelter more bright advantages of ester as drug for pharmaceutical and medicinal utilizations[23-26] .

A traditionally less common breast cancer nowadays being possibly originates over human due to industrialization and environmental factors including diet and exposure to free radicals[19-22]. The estrogen like hormones are in particular linked to the disease and promote its growth, especially diagnosed between the ages of 50 and 69 years [23]. The advent antineoplastic drugs used to treat breast cancer adjoining adverse effects like thromboembolism, cataracts and perimenopausal symptoms on selective estrogen replacement modulators and aromatase inhibitors, current therapeutic modalities are also associated with toxicity and side effects[24-27]. The current state of research on breast cancer assumed to be poor, prediction of novel therapeutic agents is needed to be developed as potential anti-tumor agents.

**Experimental section**

General All reagents for the synthesis were obtained from commercially available sources (Merck/Avra Chemical Companies) and used without any further purification. All target compounds were purified by silica gel column chromatography using Avra silica gel for 60-120 mesh. The chemical structures of the compounds were identified through $^{1}H$ and $^{13}C$ NMR spectroscopy and high-resolution mass spectrometry (MS) analyses. The FT-IR $cm^{-1}$ spectra were recorded using Agilent Technologies FT-IR (carry 630) spectrophotometer with KBr discs to get FT-IR $cm^{-1}$ spectra. The $^{1}H/^{13}C$ NMR spectra were recorded by a Bruker 400/100 MHz nuclear magnetic resonance device by dissolving the compounds in $CDCl_3$. The internal location for calculating chemical shifts (δ) in parts per million is tetramethyl silane. The internal location for calculating chemical shifts (δ) in ppm. GCMS mass spectrometry was recorded using GC-MS (sophisticated Instrument) molecular ion peak m/z.

**Synthesis of compounds 2.1-2.12**

Place 2-phenylacetic acid (compound a-e, 13.15 g), 3-methylbutan-1-ol (compound f-k, 10.88 ml), N,N'-Dicyclohexylcarbodiimide (20.63 g) and 4-Dimethylaminopyridine (12.21 g) in a 500 ml clean round-bottled flask. Add 100 ml dichloromethane in water both at $48^{0}C$ reflex with 6 hours. The reaction was completed, the reaction mixture was filtered off, then solvent was evaporated to dryness. After the reaction sample was purified by using flash column chromatography method (ethyl acetate: petroleum ether = 1:4) and the pure product (Yield: 75%) was colourless liquid. Separated isopentyl-2-phenylacetate was dried under vacuum and fully characterized by spectroscopic applications. Compounds 2.1-2.12 were prepared by the same synthesis method, all of which were colourless liquid with Yield: 70-85%.

**2.1** isopentyl 2-phenylacetate. Yield: 75%; FT-IR (carry 630) $cm^{-1}$: 1732, 3063, 3034, 2959, 2870, 1453, 1297, 1248, 1136, 1051, 976 and 693; $^{1}H$ MNR (400 MHz, $CDCl_3$) δ, ppm: 3.44 (s, 2H), 0.75 (d, 6H), 3.97 (t, 2H), 1.36 (m, 2H), 1.50 (m, 1H) and 7.0 – 7.1; $^{13}C$ NMR (400 MHz, $CDCl_3$) δ, ppm: 171.48, 134.91, 133.96, 130.88, 129.24, 128.58, 126.99, 63.48, 41.42, 37.31, 25.06 and 22.43; ESI-MS [M+H] 207.13 m/z, [M+2] 390.1060 m/z. found: 206.13; Analytical calculation for: $C_{13}H_{18}O_2$, found %: C (75.69), H (8.80), O (15.51).

**2.2** isopropyl 2-(4-methoxyphenyl)acetate. Yield: 69%; FT-IR (carry 630) $cm^{-1}$: 2981, 2937, 2836, 1725, 1613, 1584, 1513, 1464, 1297, 1174, 1030,

965 and 812; $^{1}$H MNR (400 MHz, $CDCl_3$) δ, ppm: 4.03 (t, 1H), 3.77 (s, 3H), 3.46 (s,2H), 1.42 (q, 2H), 1.56 (m, 1H) and 6.7 – 6.74; $^{13}$C NMR (400 MHz, $CDCl_3$) δ, ppm: 171.72, 148.84, 126.60, 121.33, 112.38, 111.18, 60.69, 55.73, 40.82, 29.62, 24.74 and 14.12; Analytical calculation for: $C_{12}H_{16}O_3$, found %: C (69.21), H (7.74), O (23.05).

**2.3** sec-butyl 2-(4-methoxyphenyl)acetate. Yield: 85%; FT-IR (carry 630) cm$^{-1}$: 1725, 2974, 2937, 2836, 1613, 1513, 1110, 1162, 1032, 820 and 790; $^{1}$H MNR (400 MHz, $CDCl_3$) δ, ppm: 3.60 (s, 3H), 0.77 (t, 6H), 3.43 (s, 2H), 4.77 (m, 1H), 1.46 (m, 2H), 1.10 (d, 3H) and 6.7 – 7.1; $^{13}$C NMR (400 MHz, $CDCl_3$) δ, ppm: 171.09, 158.63, 131.96, 130.79, 129.60, 128.72, 126.65, 123.19, 121.30, 114.20, 112.63, 111.38, 72.15, 40.58, 28.69, 19.21 and 9.46; ESI-MS [M+H] 207.13 m/z, [M+2] 390.1060 m/z. found: 208.11; Analytical calculation for: $C_{12}H_{16}O_3$, found %: C (69.21), H (7.74), O (23.05).

**2.4** isopentyl (2-(4-methoxyphenyl)acetate. Yield: 92%; FT-IR (carry 630) cm$^{-1}$: 3060, 3026, 2933, 2959, 2836, 2784, 1729, 1513, 1453, 1420, 1259, 1237, 1140, 1028 and 756; $^{1}$H MNR (400 MHz, $CDCl_3$) δ, ppm: 4.09 (t, 2H), 3.69 (s, 3H), 0.89 (d, 6H), 3.50 (s, 2H), 1.63 (t, 2H), 1.48 (m, 1H) and 6.7 – 7.1; $^{13}$C NMR (400 MHz, $CDCl_3$) δ, ppm: 171.63, 158.70, 134.29, 130.83, 129.20,128.43, 126.91, 114.01, 63.22, 54.89, 40.37, 37.31, 25.03 and 22.37; ESI-MS [M+H] 207.13 m/z, [M+2] 390.1060 m/z. found : 236.14; Analytical calculation for: $C_{14}H_{20}O_3$,; found %: C (71.16), H (8.53), O (20.31).

**2.5** methyl 2-(3-,4-dimethoxyphenyl)acetate. Yield: 87%; FT-IR (carry 630) cm$^{-1}$: 3060, 3026, 2933, 2959, 2836, 2784, 1729, 1513, 1453, 1420, 1259, 1237, 1140, 1028 and 756; $^{1}$H MNR (400 MHz, $CDCl_3$) δ, ppm: 4.09 (t, 2H), 3.69 (s, 3H), 0.89 (d, 6H), 3.50 (s, 2H), 1.63 (t, 2H), 1.48 (m, 1H) and 6.7 – 7.1; $^{13}$C NMR (400 MHz, $CDCl_3$) δ, ppm: 171.43. 148.79, 146.57, 121.26, 112.37, 111.15, 77.28, 60.52, 55.57, 40.64, 14.01 and 13.98; ESI-MS [M+H] 207.13 m/z, [M+2] 390.1060 m/z. found: 210.23; Analytical calculation for: $C_{11}H_{14}O_4$, found %: C (62.85), H (6.71), O (30.44).

**2.6** ethyl 2-(3,4-dimethoxyphenyl)acetate. Yield: 70%; FT-IR (carry 630) cm$^{-1}$: 2937, 2881, 2836, 1729, 1591, 1513, 1461, 1420, 1233, 1140, 1025 and 764; $^{1}$H MNR (400 MHz, $CDCl_3$) δ, ppm: 4.07 (q, 2H), 3.78 (s, 3H), 1.16 (t, 3H), 3.48 (s, 2H) and 6.72 – 6.77; $^{13}$C NMR (400 MHz, $CDCl_3$) δ, ppm: 171.72, 148.84, 126.60, 121.33, 112.38, 111.18, 60.69, 55.73, 40.82, 29.62, 24.74 and 14.12; ESI-MS [M+H] 207.13 m/z, [M+2] 390.1060 m/z. found: 224.25; Analytical calculation for: $C_{12}H_{16}O_4$, found%: C (64.27), H (7.19), O (28.54).

**2.7** isopropyl 2-(3,4-dimethoxyphenyl)acetate. Yield: 75%; FT-IR (carry 630) cm$^{-1}$: 3063, 2937, 2836, 1725, 1591, 1513, 1591, 1453, 1420, 1233, 1259, 1140, 1062 and 700; $^{1}$H MNR (400 MHz, $CDCl_3$) δ, ppm: 4.07 (m, 1H), 3.78 (s, 2H), 1.44 (d, 6H), 3.79 (s, 3H) and 7.18 – 7.24; $^{13}$C NMR (400 MHz, $CDCl_3$) δ, ppm: 171.02, 148.91, 128.47, 127.87, 126.54, 121.45, 112.32, 111.19, 60.83, 55.90, 41.21, 22.18 and 14.13; ESI-MS [M+H] 207.13 m/z, [M+2] 390.1060 m/z. found: 238.28; Analytical calculation for: $C_{13}H_{18}O_4$, found%: C (65.53), H (7.61), O (26.86).

**2.8** sec-butyl 2-(3,4-dimethoxyphenyl) acetate. Yield: 75%; FT-IR (carry 630) cm$^{-1}$: 2937, 2836, 1722, 1591, 1461, 1420, 1259, 1233, 1144, 1028, 991 and 790; $^{1}$H MNR (400 MHz, $CDCl_3$) δ, ppm: 4.65 (m, 1H), 3.47 (s, 3H), 3.30 (s,2H), 1.31 (d, 3H), 1.14 (t, 3H) and 6.9 – 6.5; $^{13}$C NMR (400 MHz, $CDCl_3$) δ, ppm:171.08, 148.75, 147.95, 130.71, 127.05, 126.98, 121.45, 120.89, 113.98, 112.61, 111.92, 110.73, 72.23, 55.51, 40.97, 28.58, 19.17 and 9.40; ESI-MS [M+H] 207.13 m/z, [M+2] 390.1060 m/z. found: 252.14; Analytical calculation for: $C_{14}H_{20}O_4$, found%: C (66.65), H (7.99), O (25.37).

**2.9** tert-butyl 2-(3,4-dimethoxyphenyl) acetate. found: 252.14; Yield: 85%; FT-IR (carry 630) cm$^{-1}$: 3063, 2959, 1725, 1592, 1463, 1430, 1244, 1164, 1023, 978, 782; $^{1}$H MNR (400 MHz, $CDCl_3$) δ, ppm: 4.07 (m, 1H) and 7.26 – 6.72; $^{13}$C NMR (400 MHz, $CDCl_3$) δ, ppm:171.08, 147.74, 130.75, 126.98, 121.45, 120.89, 113.98, 112.61, 111.92, 110.73, 72.23, 55.51, 41.21, 28.58, 19.17 and 10.21; Analytical calculation for: $C_{14}H_{20}O_4$, found%: C (66.65), H (7.99), O (25.37).

**2.10** isopentyl 2-(3,4-dimethoxyphenyl) acetate. Yield: 75%; FT-IR (carry 630) cm$^{-1}$: 2955, 2836, 1729, 1591, 1513, 1259, 1237, 1140, 1028 and 764; $^{1}$H MNR (400 MHz, $CDCl_3$) δ, ppm: 4.03 (t, 1H), 3.77 (s, 3H), 3.46 (s,2H), 1.42 (q, 2H), 1.56 (m, 1H) and 6.7 – 6.74; $^{13}$C NMR (400 MHz, $CDCl_3$) δ, ppm: 171.08, 148.75, 147.95, 130.71, 127.05, 126.98, 121.45, 120.89, 113.98, 112.61, 111.92, 110.73, 72.23, 55.51, 40.97, 28.58, 19.17 and 9.40; ESI-MS [M+H] 207.13 m/z, [M+2] 390.1060 m/z. found: 266.33; Analytical calculation for $C_{15}H_{22}O_4$, found%: C (67.64), H (8.33), O (24.03).

**2.11** isopentyl 2-(3,5-dimethoxyphenyl) acetate. Yield: 64%; FT-IR (carry 630) cm$^{-1}$: 2937, 2936, 1733, 1591, 1513, 1243, 1237, 1140, 1028 and 764; $^{1}$H MNR (400 MHz, $CDCl_3$) δ, ppm: 4.07 (t, 1H), 3.77 (s, 3H), 3.46 (s,2H), 1.42 (q, 2H), 1.56 (m, 1H) and 6.72 – 7.26; $^{13}$C NMR (400 MHz, $CDCl_3$) δ, ppm: 171.72, 148.75, 147.95, 130.71, 127.05, 126.98, 121.45, 120.89, 113.98, 112.61, 111.92, 110.73, 60.69, 55.51, 40.97, 28.58, 19.17 and 9.40;

Analytical calculation for $C_{15}H_{22}O_4$, found%: C (67.64), H (8.33), O (24.03).

**2.12** isopentyl 2-(3,4,5-trimethoxyphenyl) acetate. Yield: 78%; FT-IR (carry 630) $cm^{-1}$: 3063, 2936, 2836, 1722, 1591, 1513, 1461, 1420, 1255, 1140, 1025 and 764; $^1H$ MNR (400 MHz, $CDCl_3$) δ, ppm: 4.03 (q, 2H), 3.78 (s, 3H), 1.16 (t, 3H), 3.48 (s, 2H) and 6.78 – 7.42; $^{13}C$ NMR (400 MHz, $CDCl_3$) δ, ppm: 171.72, 148.84, 126.60, 121.33, 112.38, 111.18, 63.22, 55.73, 41.42, 29.62 and 20.31; $C_{16}H_{24}O_5$, found%: C (64.84), H (8.16), O (26.99).

**Anti-inflammatory activity:**

**BSA denaturation technique:** The synthesized compounds **2.4a, 2.11a, 2.11, 2.12a** and **standard diclofenac sodium** were screened for anti-inflammatory activity by using the inhibition of albumin denaturation technique with minor modification. The standard drug and the compounds were dissolved in minimum quantity of Dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, PH 7.4). The final concentration of DMF in all solution was less than 2.5%. Test Solution (2.5 mL) containing different concentrations of the drug was mixed with 1 mL of 1 mM Bovine serum albumin solution in phosphate buffer and incubated at 37 °C in an incubator for 10 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm. Percentage of Inhibition of denaturation was calculated from control using the following formula.

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

**Anti-cancer studies:**

Traditional *in-vitro* toxic effect determinations of synthesized drug candidates have been executed by counting viable cells after staining with a vital dye. MTT system, through which, measuring the activity of living cells is simple, accurate and always yielding best reproducible results via mitochondrial dehydrogenases, a bio-chemical process transfers two hydrogen atoms from organic compounds to electron acceptors, thereby oxidizing the organic compounds and generating energy. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity.

**Materials for MTT Assay Method:**

1. MTT Powder
2. DMSO
3. $CO_2$ incubator
4. Tecan Plate reader

**Preparation of test solutions**

The prepared solution using DMSO was filtered through a 0.2 µ m filter and stored at 2–8 °C for frequent use. For cytotoxicity studies, serial two fold dilutions from 100µM to 0 µM were prepared which is then used for treatment.

**Cell lines and culture medium**

All the cell lines were procured from ATCC, stock cells were cultured in DMEM/ F12 supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) in a humidified atmosphere of 5% $CO_2$ at 37ºC until confluent. The cell was dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells is checked and centrifuged. Further, 50,000 cells /well of Jurkatwas seeded in a 96 well plate and incubated for 24 hrs at 37ºC, 5 % $CO_2$ incubator.

**Source of reagents:** F12, DMEM, FBS, Pen-Strep, Trypsin procured from Invitrogen.

**Procedure:**

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x $10^5$ cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µl of the diluted cell suspension (50,000cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37ºC for 24hrs in 5% $CO_2$ atmosphere. After incubation the test solutions in the wells were discarded and 100 µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37º C in 5% $CO_2$ atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and the concentration of test drug needed to inhibit cell growth by 50% ($IC_{50}$) values is generated from the dose-response curves for each cell line.

$$\% \text{ Viability} = \mathbf{100}\text{-}\left(\frac{\text{OD of Control} - \text{OD of Sample}}{\text{OD of Control}}\right) \text{ x } 100$$

## RESULTS AND DISCUSSION:

Steglich esterification, which takes place at room temperature in the presence of solvent like dichloromethane, coupling reagent dicyclohexylcarbodimide (DCC) and catalyst 4-dimethylaminopyridine (DMAP) is used for the synthesis of differently substituted phenylacetates **(DSPA: 2.1-2.12)**. The synthesized drug candidates are subjected to structural characterization by IR, $^{1}$H-NMR, $^{13}$C-NMR spectral studies and the molecular weight determination with fragmental futures using Mass Spectrometry followed by its docking and bio-logical screening.

### FT-IR spectral data:

One of the most characteristic vibrational frequency peak for the synthesized ester in the spectral chart is appeared as prominent peak at 1733 cm$^{-1}$ for carbonyl group and C–O stretching peak is also detectable due to absorption at 1248 cm$^{-1}$. The aryl C–H stretching band appeared in the range 3063-3034 cm$^{-1}$ and alkyl C–H stretching in the range of 2959-2870 cm$^{-1}$ for the studied compound **2.1** the spectrum are given in **Figure 1**. all the synthesized compounds FT-IR spectral data are given in **table 1**.

**Table 1:** FT-IR spectral data for structural analysis of synthesized compounds **DSPA: 2.1-2.12**

| Entry | FT-IR (ATR) stretching frequencies (cm$^{-1}$) | | | |
|---|---|---|---|---|
| | C=O | Aro >C-H | Ali >C-H | C-O |
| **2.1** | 1733 | 3034 | 2959 | 1248 |
| **2.2** | 1725 | 2981 | 2937 | 1244 |
| **2.3** | 1725 | 2974 | 2937 | 1244 |
| **2.4** | 1729 | 3026 | 2959 | 1259 |
| **2.5** | 1729 | 3026 | 2959 | 1255 |
| **2.6** | 1729 | 2937 | 2881 | 1233 |
| **2.7** | 1725 | 3063 | 2937 | 1259 |
| **2.8** | 1722 | 2937 | 2836 | 1233 |
| **2.9** | 1725 | 3063 | 2959 | 1244 |
| **2.10** | 1729 | 3026 | 2981 | 1259 |
| **2.11** | 1733 | 2937 | 2936 | 1243 |
| **2.12** | 1722 | 3063 | 2936 | 1255 |

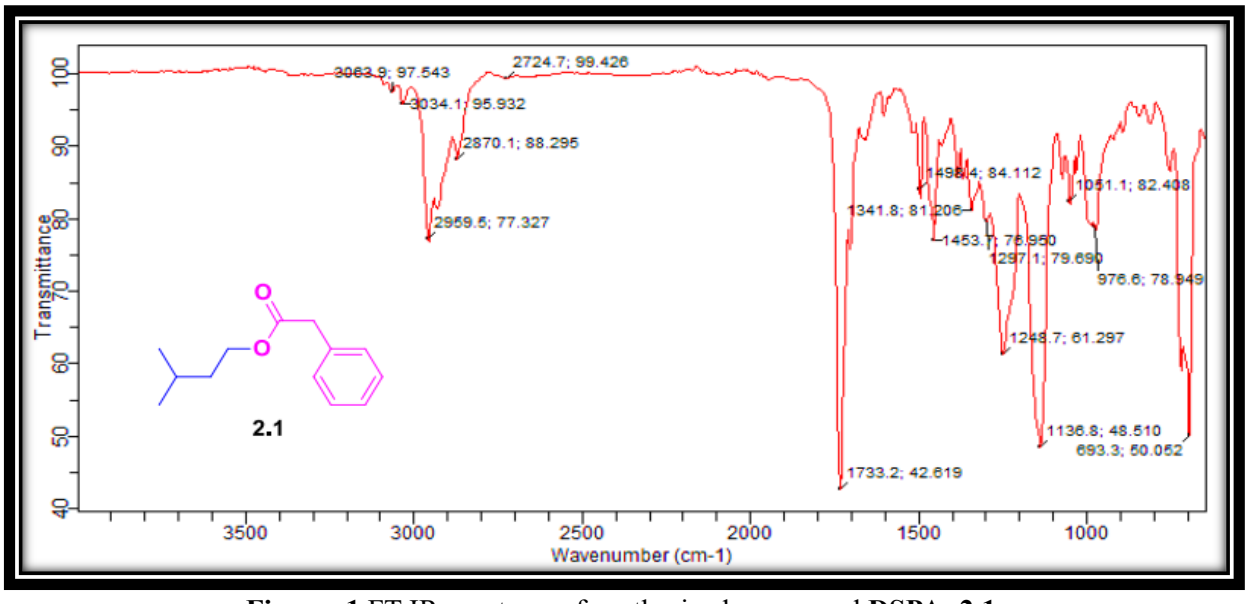


**Figure :1** FT-IR spectrum of synthesized compound **DSPA: 2.1.**

### $^{1}$H NMR spectral data:

The more informative $^{1}$H NMR spectrum recorded for **2.1** was confirmed the presence of phenyl and alkyl groups in the ester and also the structural assignment lead to a name *iso*-amyl phenylacetate. A few characteristic signals were observed at d 0.75 (d, 6H), 1.36 (m, 1H), 3.44 (m, 2H) and 3.99 (t, 2H) ppm assignable to *iso*-amyl protons and the signal in the range of 7.06-7.11 ppm was attributed to phenyl ring protons. Compound 2.1 sperum as given in **Figure 2**. Synthesized compounds (**2.1-**

**2.12**) $^{1}$H NMR spectral details are entered in **table 2.**

**Table 2**: $^{1}$H NMR spectral data for structural analysis of synthesized compounds **DSPA: 2.1-2.12.**

| Entry | $C_7$-H ppm | $C_9$-H ppm | $C_{10}$-H ppm | Aro >C-H ppm | Methyl proton ppm | Methoxy proton ppm |
|---|---|---|---|---|---|---|
| **2.1** | 3.44 (s) | 3.99 (t) | 1.36 (m) | 7.06-7.11 | 0.75 (d) | - |
| **2.2** | 3.44 (s) | - | 1.46 (m) | 7.06-7.12 | 0.76 (d) | - |
| **2.3** | 3.43 (s) | 4.77 (m) | 1.46 (m) | 6.74-7.12 | 0.77 (t) | 3.60 (s) |
| **2.4** | 3.50 (s) | 4.09 ( | 1.63 (t) | 6.80-7.42 | 0.89 (d) | 3.69 (s) |
| **2.5** | 3.65 (s) | 4.07 (t) | 3.47 (s) | 6.60-6.69 | - | 3.95 (s) |
| **2.6** | 3.98 (s) | 4.07 (m) | - | 6.72-6.77 | 1.16 (d) | 3.78 (s) |
| **2.7** | 3.78 (s) | 4.07 (m) | 1.43 (t) | 7.18-7.24 | 1.44 (d) | 3.79 (s) |
| **2.8** | 3.33 (s) | 4.65 (m) | - | 6.57-6.99 | 1.28 (t) | 3.47 (s) |
| **2.9** | - | 4.07 (m) | - | 6.72-7.26 | - | - |
| **2.10** | 3.46 (s) | 4.03 (t) | 1.42 (m) | 6.72-6.74 | 0.81 (d) | 3.77 (s) |
| **2.11** | - | 4.07 (t) | 1.53 (t) | 6.60-7.26 | - | - |
| **2.12** | - | 4.03 (m) | - | 6.78-7.42 | - | - |

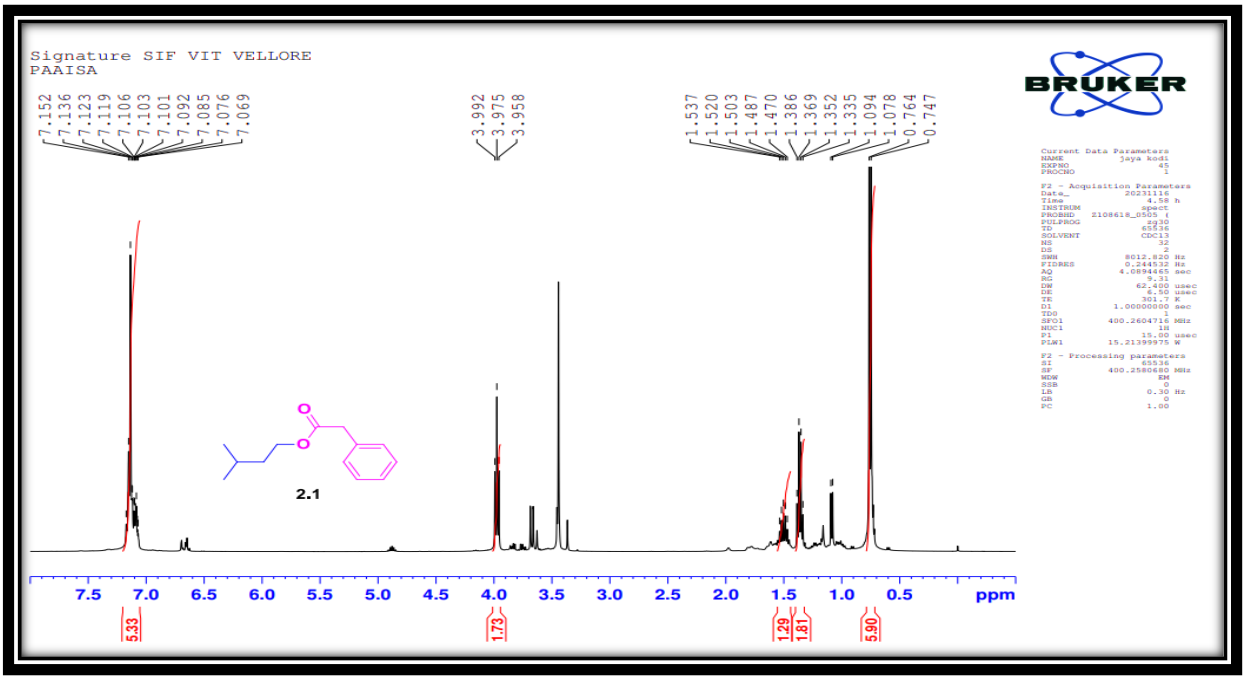


**Figure :2** $^{1}$H NMR spectrum of synthesized compound **DSPA: 2.1.**

**$^{13}$C NMR spectral data:**

The recorded $^{13}$C-NMR spectrum of **2.1** using $CDCl_3$ as solvent displayed the following signals at δ 22.43, 25.06, 37.31, 41.42 and 63.48 are assigned to iso-amyl group. A few characteristic signals (2.1-2.12) $^{13}$C NMR data are given **table 3.** appeared in the range of δ 126-134 are belongs to phenyl ring and signal at δ 171.48 is characteristic for carbonyl carbon, the results of $^{13}$C-NMR in total confirmed the assigned structure of **2.1** and the spectral image are given in **Figure 3**. Compound

**Table 3**: $^{13}$C NMR spectral data for structural analysis of synthesized compounds **DSPA: 2.1-2.12.**

| Entry | C=O ppm | $C_7$ ppm | $C_9$ ppm | $C_{10}$ ppm | $C_{11}$ ppm | Methyl carbon ppm | Aro >Carbon ppm | Methoxy carbon ppm |
|---|---|---|---|---|---|---|---|---|
| **2.1** | 171.48 | 63.48 | 41.42 | 25.06 | 37.31 | 22.43 | 126-134 | - |
| **2.2** | 171.82 | - | 40.21 | 20.31 | - | - | 121-131 | - |
| **2.3** | 171.09 | 72.15 | 40.58 | 28.69 | - | 19.21 | 111-131 | 54.73 |
| **2.4** | 171.63 | 63.22 | 40.37 | 37.31 | 22.37 | - | 113-134 | 54.89 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **2.5** | 171.43 | 60.43 | - | 27.97 | - | 13.98 | 111-126 | 55.54 |
| **2.6** | 171.72 | 60.69 | 40.82 | - | - | 14.12 | 111-126 | 55.73 |
| **2.7** | 171.02 | 60.83 | 41.21 | 20.41 | - | 14.13 | 111-128 | 55.90 |
| **2.8** | 171.08 | 72.23 | 40.97 | 19.17 | 28.58 | 9.40 | 110-130 | 55.51 |
| **2.9** | 171.08 | - | 41.21 | - | - | 10.21 | 110-131 | - |
| **2.10** | 171.02 | 72.15 | 40.21 | - | - | 14.31 | 113-133 | - |
| **2.11** | 171.72 | 60.69 | - | - | - | - | 120-128 | - |
| **2.12** | 171.72 | 63.22 | 41.42 | - | - | 20.31 | 121-130 | - |

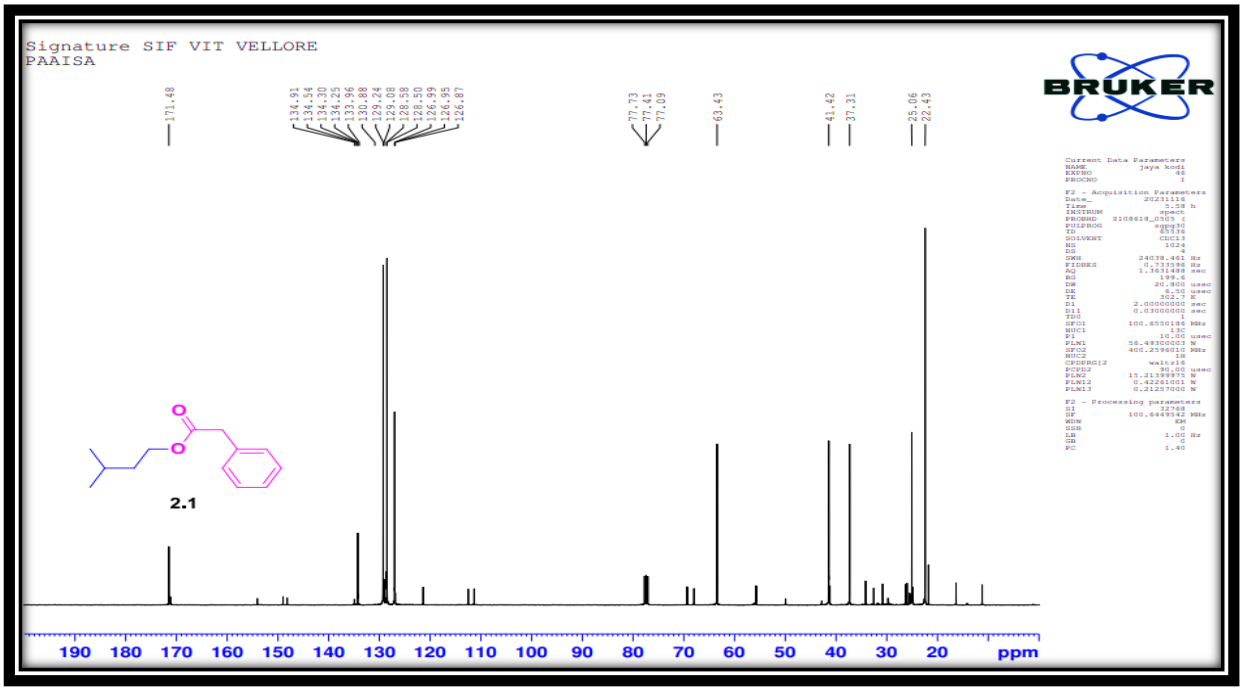


**Figure :3** $^{13}$C NMR spectrum of synthesized compound **DSPA: 2.1**

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**Mass spectrometry**

The recorded mass spectrometry of **2.1** using methanol as solvent displayed the following molecular ion peak present the m/z value of synthesized compound 2.1 is theoretically calculated and the appeared [M+1] peak is 206.13 m/z and the spectrometry data was given in **Figure 4**. Mass spectrometry data are given **table 4.**

**Table 4**: Mass spectrometry data for structural analysis of synthesized compounds **DSPA: 2.1-2.10.**

| Entry | [M+1] peak m/z |
|---|---|
| **2.1** | 206.13 |
| **2.2** | 208.11 |
| **2.3** | 222.13 |
| **2.4** | 236.14 |
| **2.5** | 210.09 |
| **2.6** | 224.10 |
| **2.7** | 238.12 |
| **2.8** | 252.14 |
| **2.9** | - |
| **2.10** | 266.15 |

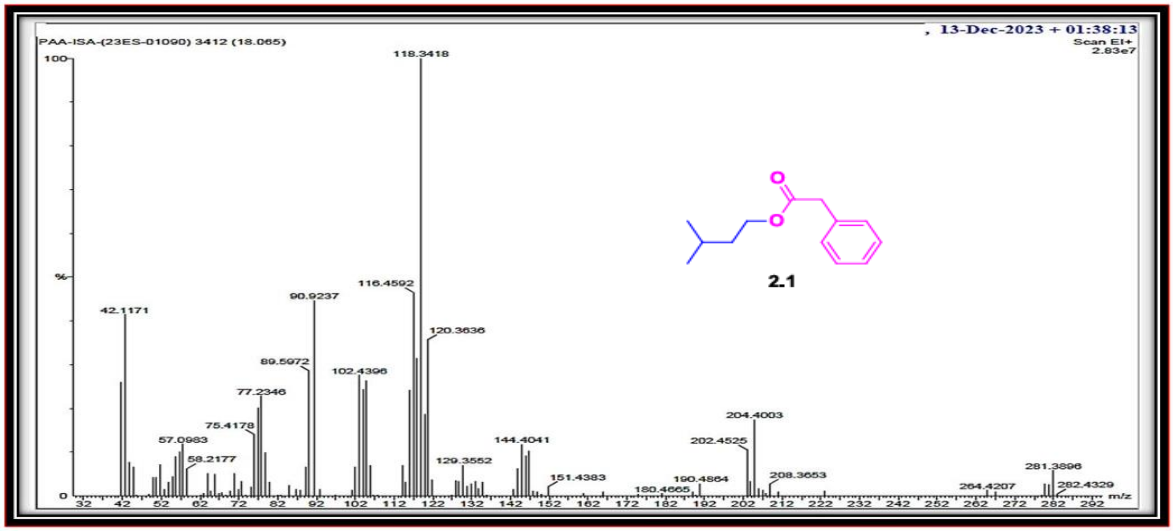


**Figure :4** Mass spectrometry spectrum of synthesized compound **DSPA: 2.1**

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**Molecular docking studies:**

Molecular docking studies were carried out for the better understanding of binding affinity between potent inhibitors (**2.1.-2.12.**) and the binging-site of the receptors, when interacting with the protein. Herewith, we have chosen three different proteins by trial-and-error method, viz. analgesic (**5du1**), anti-inflammatory (**5glw**) and anti-breast cancer active protein (**6fe2**) and then downloaded from protein data bank, glide grid generated for the receptor and molecular docking of the receptor with our hit compounds (**2.1.-2.12.**) were performed.

The geometrical coordinates obtained from single crystal X-ray crystallography of the proteins have been utilized for docking studies. The three dimensional structures of the ligands **2.1.-2.12.** were obtained from Open Babel software and energy minimization of the structure performed using AMBER force field. All these obtained structures were converted into pdbqt files (required for docking) using MGL Tools after adding hydrogens and fixing formal charges. In the same way, the protein structures (PDB ID: **5du1**, **5glw** and **6fe2**) were also prepared for the docking studies. The docking of the protein and every individual compound was performed using 120 × 120 × 120 grid box through Lamarckian Genetic Algorithm with the help of AutoDock 4.2 application. Prepared compound (2.1-2.12) analogs are treated with different types of proteins and the binding energy scores are entered in **table 5**. Synthesised compound 2.1 docking 2D and 3D image are given in **Figure 5**.

**Table 5**: Molecular docking results (binding energy) of compounds **DSPA: 2.1-2.12**.

| Entry | Binding energy (kcal/mol) Against Protein ID | | |
|---|---|---|---|
| | 5du1 (Analgesic) | 5glw (Anti-Inflammatory) | 6fe2 (Anti-Breast cancer) |
| **2.1** | **-4.76** | **-3.12** | **-4.52** |
| **2.2** | -4.54 | -3.78 | -5.31 |
| **2.3** | **-4.91** | **-3.97** | -4.55 |
| **2.4** | **-4.71** | **-3.73** | **-5.10** |
| **2.5** | -4.29 | -3.52 | **-5.80** |
| **2.6** | -4.31 | -2.93 | -5.78 |
| **2.7** | -4.34 | -3.24 | -5.26 |
| **2.8** | -4.41 | -2.09 | -5.61 |
| **2.9** | -4.68 | -3.16 | -4.46 |
| **2.10** | **-4.73** | **-2.82** | **-5.28** |
| **2.11** | **-4.87** | **-2.97** | **-5.71** |
| **2.12** | **-4.73** | **-3.06** | **-4.55** |

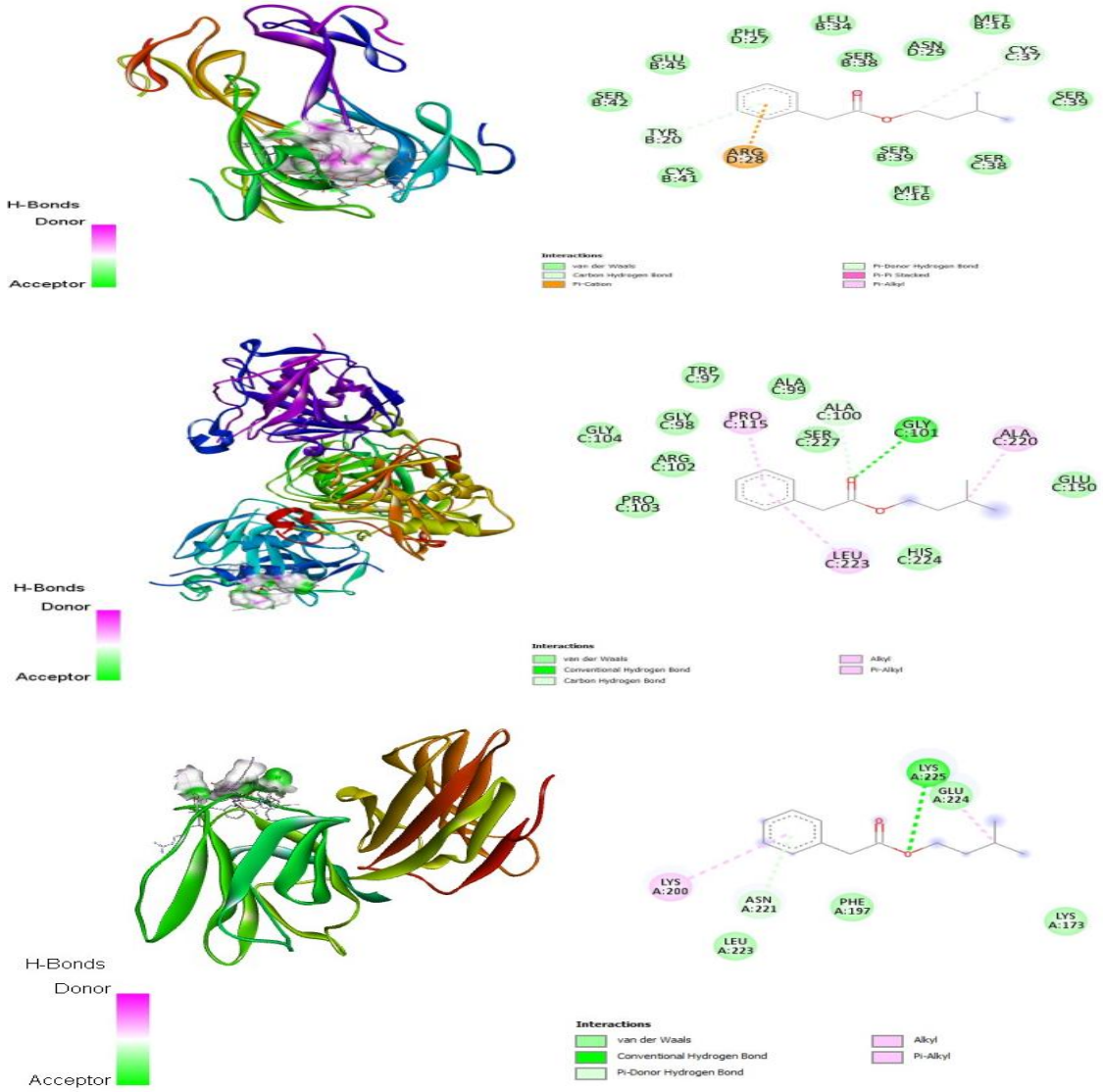



**Figure :5** 3D and 2D docking image of synthesized compound **DSPA: 2.1.**

Firstly, **5du1** PDB code a crystal structure of dendroaspis polylepis mambalgin-1 with acid-sensing ion channels (ASICs) to **relieve pain** is used for docking study. Crystal structure of dendroaspis polylepis mambalgin-1 a pain-relieving peptide and functional domain for inhibition of acid-sensing ion channels (ASICs), that could have therapeutic value against pain and also probably other **neurological disorders**. The well-designed active site by which the toxin inhibits ASIC channels was recognized in its loop II and more exactly in the face comprising Phe-27, Leu-32, and Leu-34 residues. Moreover, proximity between Leu-32 in mambalgin-1 and Phe-350 in rASICs was proposed from double mutant cycle analysis[28].

The virtual screening results obtained from AutoDock 4.2 application of **5du1** against the synthesized ligands were displayed very good binding energy ranges from **-4.29** kcal/mol (Methyl 3,4-dimethoxyphenylacetate) to **-4.91** kcal/mol **(**2-Butyl 4-methoxyphenylacetate), an indication for being as potential drug candidates. Surprisingly, unaffected analgesic effects (Binding energy variations are only from -4.73 to -4.76 kcal/mol) were observed against the number of hydrogen-bond acceptors, when increased by number of methoxy group. Among the synthesized compounds **DSPA: 2.1-2.12,** the lead optimization results support the drug candidate 2-Butyl 4-methoxyphenylacetate (**2.3**) with binding energy -4.76 kcal/mol may possess very impressive anti-analgesic effect.

Thereafter, Toxascaris leonina galectin (Tl-gal) is a galectin-9 homologue protein isolated from an adult worm of the canine gastrointestinal nematode parasite, and Tl-gal-vaccinated challenge can

inhibit inflammation in inflammatory bowel disease-induced mice. The first X-ray structure determined of Tl-gal complexes with carbohydrate N-acetyllactosamine (**5glw**) was found with formed bonds on concave surfaces of both carbohydrate recognition domains (CRDs) in Tl-gal. The idea of the binding locations HXXXR and WGXEER for recognizing carbohydrate binding is with charged critical amino acids Arg61/Arg196 and Glu80/Glu215 on the conserved motif of Tl-gal N-terminal CRD and C-terminal CRD, and the residues can affect protein folding and structure. The polar amino acids His, Asn, and Trp are also important residues for the interaction with carbohydrates through hydrogen bonding. This structural information is expected to elucidate the carbohydrate recognition mechanism of Tl-gal and improve our understanding of **anti-inflammatory mediators and modulators of immune response**[29].

Docking analysis of anti-inflammatory protein (**5glw**) against the drug candidates **DSPA: 2.1-2.12** were carried out and the results obtained in terms of binding affinities are as shown in Table 5. The binding score for the new ligands **DSPA: 2.1-2.12** ranges from -2.82 kcal/mol (*iso*-amyl 3,4-dimethoxyphenylacetate) to -3.97 kcal/mol **(**2-Butyl 4-methoxyphenylacetate) and the same for different in number methoxy group substitution ranges from -2.82 kcal/mol **(***iso*-amyl 3,4-dimethoxyphenylacetate) to -3.73 kcal/mol (*iso*-amyl 4-methoxyphenylacetate). Unlike the previous case (5du1 vs ligands) different scoring against number of methoxy groups were obtained in which iso-amyl 4-methoxyphenylacetate displayed with a good binding affinity -3.73 kcal/mol. However, a highest binding affinity score of -3.97 kcal/mol shown by 2-Butyl 4-methoxyphenylacetate (2.3.) against **5glw** which is comparatively a low score against analgesic protein **5du1.**

At lost, **6fe2** [47] an 3D-structure of a human carbonic anhydrase (CA) IX has emerged as promising anticancer target and diagnostic biomarker for solid hypoxic tumors. Novel fluorinated CA IX inhibitors exhibited up to 50 pM affinities towards the recombinant human CA IX, selectivity over other CAs, and direct binding to Zn(II) in the active site of CA IX inducing novel conformational changes as determined by X-ray crystallography. Hypoxia-induced extracellular acidification was significantly reduced in HeLa, H460, MDA-MB-231, and A549 cells exposed to the compounds, with the $IC_{50}$ values up to 1.29 nM. A decreased clonogenic survival was observed when hypoxic H460 3D spheroids were incubated with our lead compound and therefore these novel compounds can be as promising agents for CA IXspecific therapy[30].

Interestingly, the docking results of the synthesized **DSPA: 2.1-2.12** with anti-breast cancer protein **6fe2** were provided a healthier binding affinity than the above two proteins. In this study, the docking results were ranges from -4.46 kcal/mol for *tertiary*-butyl 3,4-dimethoxyphenylacetate (**2.9**) to -5.80 kcal/mol for Methyl 3,4-dimethoxyphenylacetate (**2.5**). The hydrogen-bond acceptors in phenyl group increased by number of methoxy group were also effective in terms of affinity and the *iso*-amyl 3,5-dimethoxyphenylacetate (**2.11**) revealed a positive data for further research findings. Hence, binding free energy of compound **2.5** at the binding site against **6fe2** fond to be **-5.80** Kcal/mol, may be a potiential drug candidate of the study.

Therefore, ongoing study on docking data analysis revealed that the drug candidates **2.3** and **2.5** are potentially inhibits anti-analgesic/anti-inflammatory proteins **5du1/5glw** and anti-breast cancer protein **6fe2** respectively are useful compounds for the prevention of the diseases.

Since the number of hydrogen bond donors in a molecule is found to alter the bio-transformational activities on target proteins, successions of a series of hybrid molecules like mono-, di- and tri-methoxy ester analogues have been synthesized for studies in order to compare with its corresponding carboxylic acids, in which the system possess one more hydrogen bond acceptor. The docking results Table 5 of ester (**2.1, 2.4, 2.10, 2.11,** and **2.12**) and its corresponding acids (**2.1a, 2.4a, 2.10a, 2.11a,** and **2.12a**) are evidenced the above discussion through its data are given in table 6 and the 2.1a docking results are given in Figure 6. In one case, the anti-inflammatory protein **5glw** in its docking results with the ligands shows that the acid drug candidates are more promisingly potent than the corresponding esters. However, in another case, the anti-breast cancer protein **6fe2** in its docking results with the ligands shows that the ester drug candidates are more promisingly potent than the corresponding acids and esters seems to be useful compounds for the prevention of the cancer diseases.

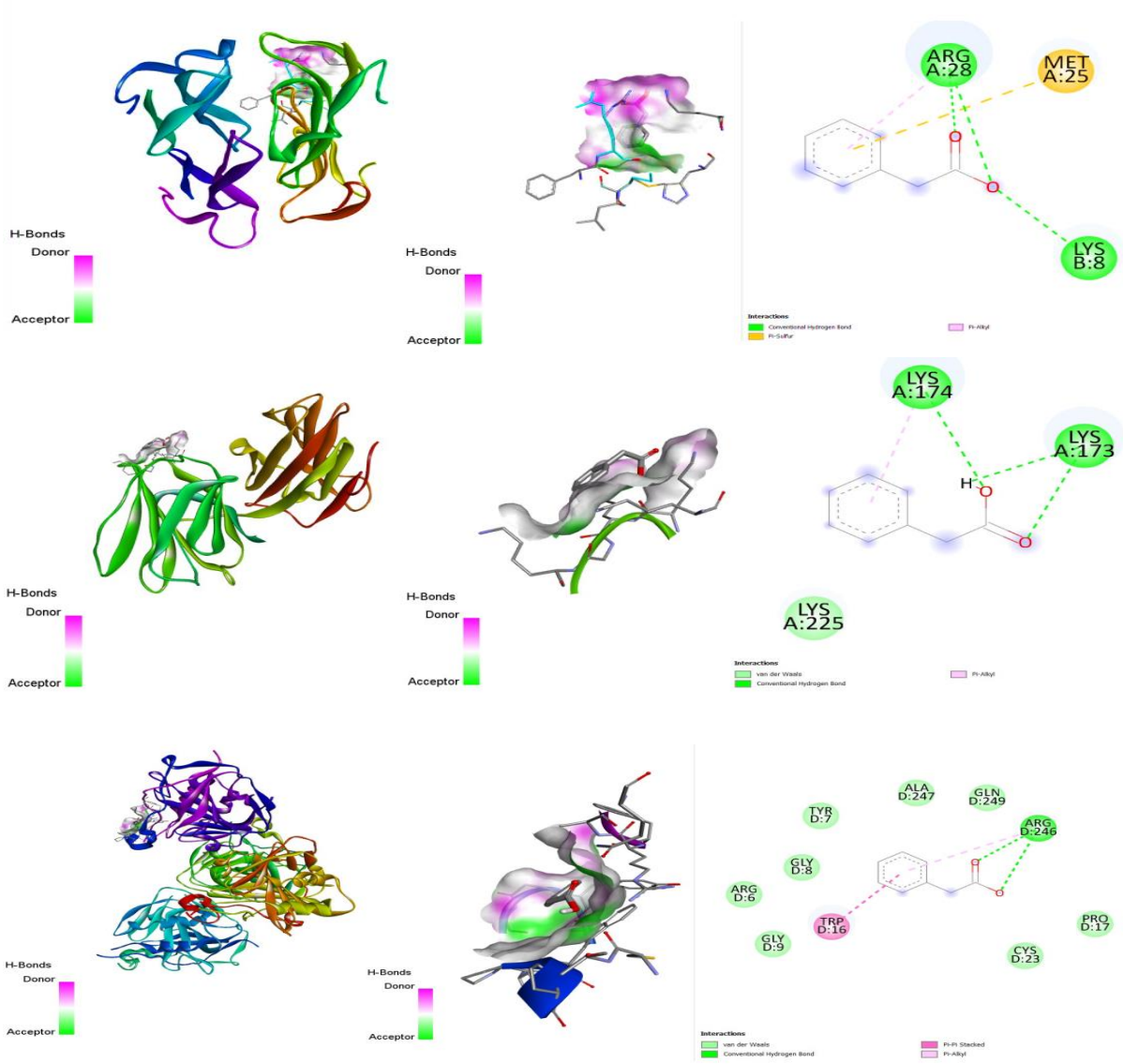



**Figure 6**: 3D and 2D image of parent compound **2.1a** docking results.

**Table 6**: Comparative docking results of the synthesized esters and its parental acids.

| Entry | Binding Energy (kcal/mol) | | | Entry | Binding Energy (kcal/mol) | | |
|---|---|---|---|---|---|---|---|
| | 5DU1 | 5GLW | 6FE2 | | 5DU1 | 5GLW | 6FE2 |
| **2.1a** | **-6.64** | **-4.15** | **-4.43** | **2.1** | **-4.76** | **-3.12** | **-4.52** |
| **2.4a** | **-6.92** | **-4.04** | **-4.64** | **2.4** | **-4.71** | **-3.73** | **-5.10** |
| **2.10a** | **-6.41** | **-3.83** | **-4.44** | **2.10** | **-4.73** | **-2.82** | **-5.28** |
| **2.11a** | **-7.34** | **-3.82** | **-4.37** | **2.11** | **-4.87** | **-2.97** | **-5.71** |
| **2.12a** | **-6.47** | **-4.16** | **-4.37** | **2.12** | **-4.73** | **-3.06** | **-4.55** |

**Anti-inflammatory activity:**

Drug candidates synthesized (**2.4a, 2.11a, 2.11, 2.12a**) and standard **diclofenac sodium** were screened for anti-inflammatory activity using the inhibition of albumin denaturation technique and the results obtained are summarized in **Table 7** and **Figure 7**. The results from the docking analysis and current evidences from the experimental data suggest that the acids (**2.4a, 2.11a and 2.12a**) are effective in reducing levels of inflammation than that of ester (**2.11**). The % of inhibitions at a concentration of 400 µg/ml are found to be 76.6565, 78.8991, 74.5158, 79.8165 and 82.2630 for **2.4a, 2.11a, 2.11, 2.12a** and **diclofenac sodium** respectively, with a biological anti-inflammatory activity order of **2.11< 2.4a < 2.11a < 2.12a < diclofenac sodium**. Although, all the synthesized

ligands have shown a very impressive anti-inflammatory activity, drug candidate **2.12a** with three methoxy group in its structure has displayed overwhelming anti-inflammatory effect when compared with the standard **diclofenac sodium.**

**Table 7**: Data of anti-inflammatory activity of synthesized compounds and standard diclofenac sodium.

| Concentration (µg/mL) | % of Inhibition | | | | |
|---|---|---|---|---|---|
| | 2.4a | 2.11a | 2.11 | 2.12a | Standard |
| 20 | 15.39245668 | 16.41182 | 14.475025 | 18.2466871 | 21.2028542 |
| 40 | 23.44546381 | 25.58614 | 21.202854 | 28.7461774 | 31.1926606 |
| 80 | 35.37206932 | 36.69725 | 32.313965 | 39.6534149 | 42.3037717 |
| 200 | 55.55555556 | 56.7788 | 53.414883 | 58.6136595 | 61.2640163 |
| 400 | 76.65647299 | 78.89908 | 74.5158 | 79.8165138 | 82.2629969 |

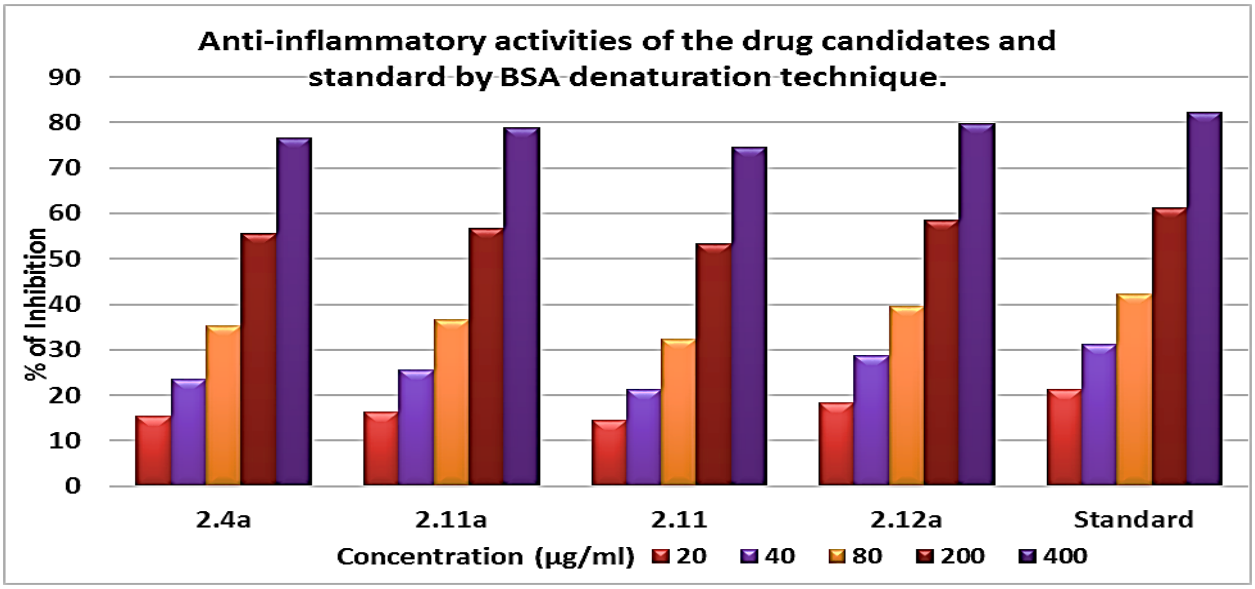



**Figure 7**: Anti-inflammatory activities of the drug candidates and standard by BSA denaturation technique.

**Anti-cancer studies:**

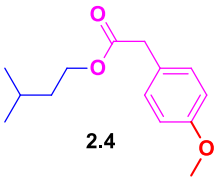
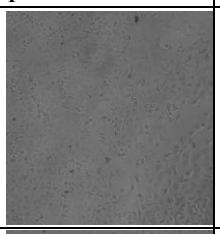
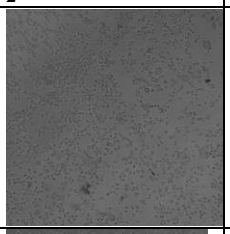
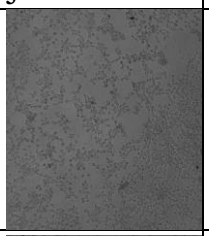
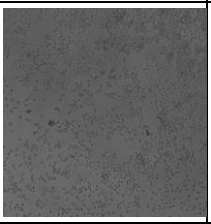
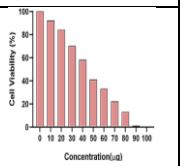
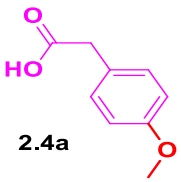
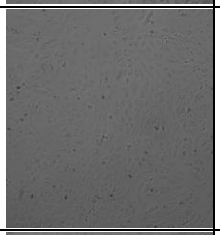
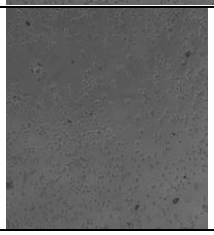
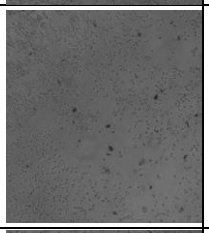
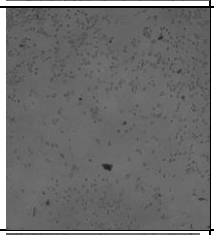
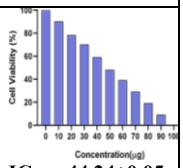
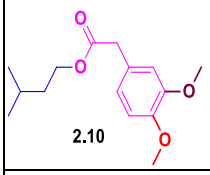
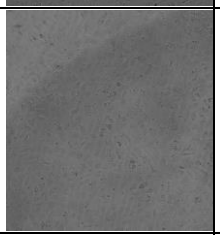
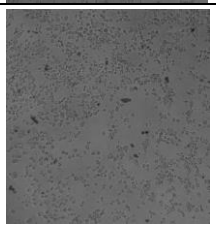
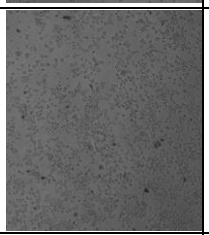
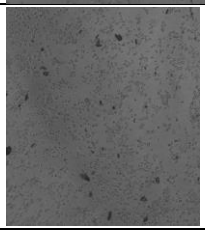
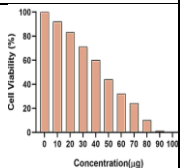
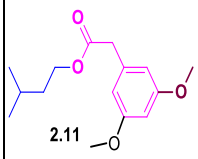
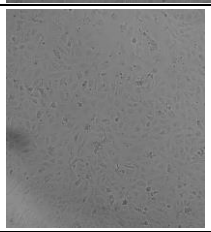
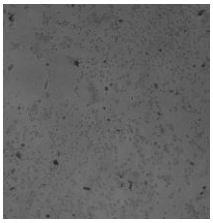
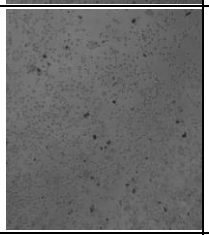
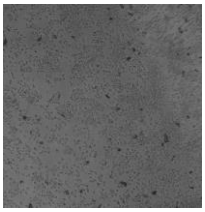
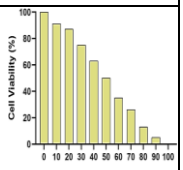
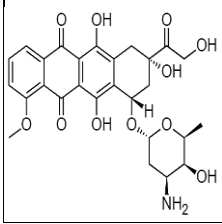
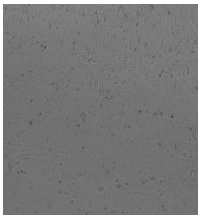
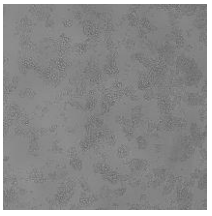
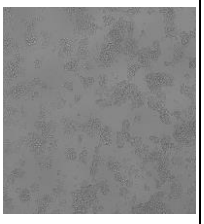
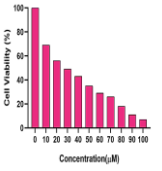
Traditional *in-vitro* toxic effect determinations of synthesized drug candidates have been executed by counting viable cells after staining with a vital dye. MTT system, through which, measuring the activity of living cells is simple, accurate and always yielding best reproducible results via mitochondrial dehydrogenases, a bio-chemical process transfers two hydrogen atoms from organic compounds to electron acceptors, thereby oxidizing the organic compounds and generating energy. The vital component 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide or MTT, is a water-soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol). The amount of resultant purple solution is then read using spectrophotometric plate reader. An increase or decrease in cell number results in an associated change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. Microplate Photometer Absorption Values at a Wavelength of 590 nm and Calculated $IC_{50}$ data are tableted in **Table 8.** Drug candidates synthesized (**2.4, 2.4a, 2.10, 2.11**) and standard **doxorubicin** were screened for anti-breast cancer activity using **MDA-MB-231** (commonly used to model late-stage breast cancer) cell lines and the results obtained are summarized in **Table 9**. The $IC_{50}$ values of the drug candidates **2.4, 2.4a, 2.10,**

**2.11** and standard **doxorubicin** were found to be 42.52± 0.05, 44.24± 0.05, 43.37± 0.05, 46.64± 0.05 and 29.27 ± 0.12 µg/ml respectively. As the observations made in the docking studies, ester drug candidate **2.4** is more promisingly potent than the corresponding acid **2.4a** and esters like **2.4** and **2.10** seems to be useful compounds for the prevention of the cancer diseases. Even though, the synthesized ligands have shown a very impressive anti-breast cancer activity, drug candidate **2.4** with para-methoxy phenyl group in its structure has been displayed devastating anti-inflammatory effect when compared with the standard **doxorubicin.**

**Table 8**: Microplate Photometer Absorption Values at a Wavelength of 590 nm and Calculated $IC_{50}$.

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **2.4** | **Blank** | **0** | **10** | **20** | **30** | **40** | **50** | **60** | **70** | **80** | **90** | **100** |
| | 0.022 | 1.006 | 0.925 | 0.845 | 0.705 | 0.578 | 0.413 | 0.332 | 0.224 | 0.134 | 0.004 | 0.001 |
| | 0.027 | 1.008 | 0.924 | 0.844 | 0.704 | 0.587 | 0.415 | 0.336 | 0.225 | 0.132 | 0.004 | 0.001 |
| | 0.027 | 1.008 | 0.925 | 0.845 | 0.705 | 0.587 | 0.418 | 0.331 | 0.227 | 0.133 | 0.008 | 0.002 |
| | **Average** | 1.007 | 0.925 | 0.845 | 0.705 | 0.584 | 0.415 | 0.333 | 0.225 | 0.133 | 0.005 | 0.001 |
| | **Inhibition %** | **0** | **8** | **16** | **30** | **42** | **59** | **67** | **78** | **87** | **99** | **100** |
| | **Viability** | **100** | **92** | **84** | **70** | **58** | **41** | **33** | **22** | **13** | **1** | **0** |
| | **IC50 = 42.52± 0.05 µg** | | | | | | | | | | | |
| **2.4a** | **Blank** | **0** | **10** | **20** | **30** | **40** | **50** | **60** | **70** | **80** | **90** | **100** |
| | 0.042 | 1.052 | 0.946 | 0.824 | 0.738 | 0.623 | 0.504 | 0.411 | 0.308 | 0.204 | 0.091 | 0.001 |
| | 0.034 | 1.054 | 0.943 | 0.823 | 0.736 | 0.622 | 0.509 | 0.411 | 0.304 | 0.202 | 0.093 | 0.001 |
| | 0.039 | 1.054 | 0.946 | 0.824 | 0.736 | 0.622 | 0.504 | 0.414 | 0.308 | 0.202 | 0.093 | 0.001 |
| | **Average** | 1.053 | 0.945 | 0.824 | 0.737 | 0.622 | 0.506 | 0.412 | 0.307 | 0.203 | 0.092 | 0.001 |
| | **Inhibition %** | **0** | **10** | **22** | **30** | **41** | **52** | **61** | **71** | **81** | **91** | **100** |
| | **Viability** | **100** | **90** | **78** | **70** | **59** | **48** | **39** | **29** | **19** | **9** | **0** |
| | **IC50 = 44.24± 0.05 µg** | | | | | | | | | | | |
| **2.10** | **Blank** | **0** | **10** | **20** | **30** | **40** | **50** | **60** | **70** | **80** | **90** | **100** |
| | 0.023 | 0.999 | 0.921 | 0.833 | 0.708 | 0.633 | 0.442 | 0.324 | 0.235 | 0.104 | 0.012 | 0.001 |
| | 0.025 | 0.998 | 0.921 | 0.834 | 0.708 | 0.635 | 0.432 | 0.325 | 0.235 | 0.104 | 0.011 | 0.001 |
| | 0.025 | 0.999 | 0.922 | 0.833 | 0.707 | 0.533 | 0.431 | 0.324 | 0.235 | 0.105 | 0.012 | 0.002 |
| | **Average** | 0.999 | 0.921 | 0.833 | 0.708 | 0.600 | 0.435 | 0.324 | 0.235 | 0.104 | 0.012 | 0.001 |
| | **Inhibition %** | **0** | **8** | **17** | **29** | **40** | **56** | **68** | **76** | **90** | **99** | **100** |
| | **Viability** | **100** | **92** | **83** | **71** | **60** | **44** | **32** | **24** | **10** | **1** | **0** |
| | **IC50 = 43.37± 0.05 µg** | | | | | | | | | | | |
| **2.11** | **Blank** | **0** | **10** | **20** | **30** | **40** | **50** | **60** | **70** | **80** | **90** | **100** |
| | 0.022 | 0.998 | 0.905 | 0.863 | 0.748 | 0.633 | 0.502 | 0.421 | 0.317 | 0.129 | 0.047 | 0.004 |
| | 0.023 | 0.998 | 0.904 | 0.863 | 0.746 | 0.632 | 0.506 | 0.311 | 0.227 | 0.129 | 0.047 | 0.004 |
| | 0.023 | 0.996 | 0.903 | 0.863 | 0.747 | 0.632 | 0.501 | 0.311 | 0.227 | 0.127 | 0.047 | 0.005 |
| | **Average** | 0.997 | 0.904 | 0.863 | 0.747 | 0.632 | 0.503 | 0.348 | 0.257 | 0.128 | 0.047 | 0.004 |
| | **Inhibition %** | **0** | **9** | **13** | **25** | **37** | **50** | **65** | **74** | **87** | **95** | **100** |
| | **Viability** | **100** | **91** | **87** | **75** | **63** | **50** | **35** | **26** | **13** | **5** | **0** |
| | **IC50 = 46.64± 0.05 µg** | | | | | | | | | | | |
| **Standard** | **Blank** | **0** | **10** | **20** | **30** | **40** | **50** | **60** | **70** | **80** | **90** | **100** |
| | 0.050 | 1.124 | 0.752 | 0.645 | 0.697 | 0.645 | 0.443 | 0.315 | 0.352 | 0.163 | 0.102 | 0.063 |
| | 0.046 | 1.345 | 0.731 | 0.624 | 0.497 | 0.341 | 0.364 | 0.378 | 0.302 | 0.294 | 0.164 | 0.102 |
| | 0.064 | 1.024 | 0.915 | 0.702 | 0.512 | 0.512 | 0.402 | 0.315 | 0.246 | 0.164 | 0.102 | 0.074 |
| | **Average** | 1.164 | 0.799 | 0.657 | 0.569 | 0.499 | 0.403 | 0.336 | 0.300 | 0.207 | 0.123 | 0.080 |
| | **Mean** | **0** | **31** | **44** | **51** | **57** | **65** | **71** | **74** | **82** | **89** | **93** |
| | **Viability** | **100** | **69** | **56** | **49** | **43** | **35** | **29** | **26** | **18** | **11** | **7** |
| | **$IC_{50}$ = 29.27 ± 0.12 µg/ml** | | | | | | | | | | | |

**Table 9**: Graph Representing the MDA-MB-231 Cell Viability on Treatment With Drug Candidates.

| Entry | Treated MDA-MB-231 cells | | | | Control cells |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
|  2.4 |  |  |  |  |  $IC_{50}$ = 42.52±0.05 µg |
|  2.4a |  |  |  |  |  $IC_{50}$ = 44.24±0.05 µg |
|  2.10 |  |  |  |  |  IC50 = 43.37± 0.05 µg |
|  2.11 |  |  |  |  |  $IC_{50}$ = 46.64±0.05 µg |
| **Standard**  |  |  |  |  |  $IC_{50}$ = 29.27 ± 0.12 µg/ml |

## CONCLUSION

Bio-Inhibitors like covalent organic framework of differently substituted alkyl phenylacetates (**DSPA: 2.1-2.12)** synthesized to control microbial toxicities and other budding threats were with high level potential. The structures of the synthesized drug candidates were characterized through IR, $^{1}$H-NMR, $^{13}$C-NMR spectral data and the molecular weight determination with fragmental futures using Mass Spectrometry. After proper validation of the docking protocol, the *in-silico* analysis of different disease condition like anti-analgesic, anti-inflammatory and anti-cancer targets (PDB ID: **5du1**, **5glw** and **6fe2**) against the synthesized compounds **DSPA: 2.1-2.12** and its corresponding acids investigated by AutoDock 4.2 application with a wider scope in the drug development.

The most useful expectations generated by in-silico modellings were correlated with experimental endorsements of the anti-inflammatory and anti-cancer drug development results. In this study it's concluded that the synthesized ester as well as its corresponding acids having their own biological activity and found one is more active than the other depending on the case. In the case of anti-inflammatory activity, results from the docking analysis and evidences from the experimental data suggest that the acids (**2.4a, 2.11a** and **2.12a**) are effective in reducing levels of inflammation than that of ester (**2.11**). However, in the anti-breast cancer activity ester drug candidate **2.4** is more promisingly potent than the corresponding acid **2.4a** and esters like **2.4** and **2.10** seems to be useful compounds for the prevention of the cancer diseases. The above innovative investigations in the concept of pro-drug and masking technology may considerably facilitate the anti-inflammatory and anti-cancer drug discovery and its development.

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