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DIURETIC EFFECT OF NOVEL STRUCTURAL ANALOGUES OF ETHACRYNIC ACID

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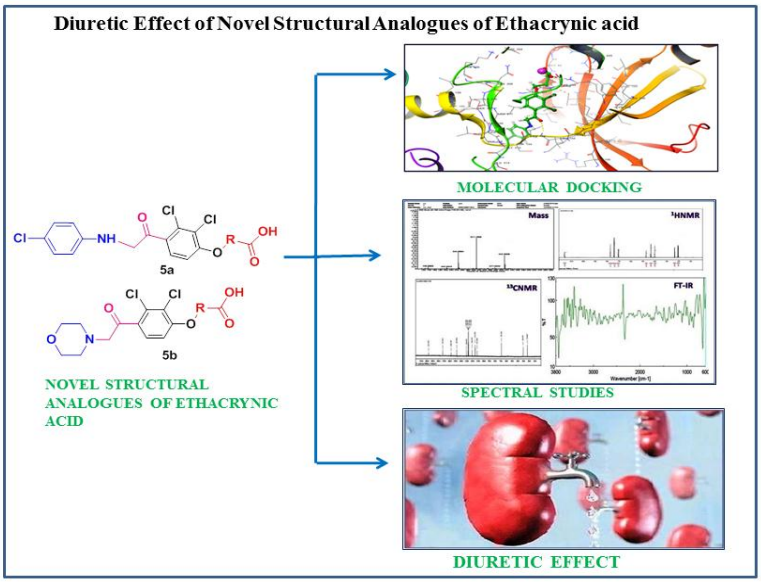
ABSTRACT
Ethacrynic acid is a highly effective clinically used loop diuretic. Literature reveals that it can produce diuresis in patients with chronic renal failure. The present research study aims to develop novel structural analogues of ethacrynic acid having greater diuretic effect. Designed compounds were docked against NKCC2 (PDB: 5DBX) using Ethacrynic acid as standard. After evaluation of docking results compounds were slected for synthesis. Synthesis was completed in three steps with the Convenient synthetic route as shown in the scheme. The Structures of novel synthesized molecules were confirmed by spectral characterization such as FTIR, ^{1}HNMR, ^{13}CNMR and Mass spectrometry. Toxicological and ADME studies were done to ensure safety and drug like properties of the novel compounds.Ten structural analogues of ethacrynic acid were synthesized and evaluated for diuretic effect in albino wistar rats. The diuretic effect was measured by calculating different parameters such as urine volume, urinary electrolyte levels (Na, K, Cl ions mmol/lit), urine pH, and conductivity. Diuretic index was calculated to correlate the diuretic potential of novel synthesized compounds. Compounds **C1, C3, C4, C5, C6** have maximum docking score as well as found with higher diuretic index/ diuretic action.We have succeeded in developing structural analogues of ethacrynic acid. All the Compounds were found with diuretic activity. Compound **C3, C5, C6,** were found with higher diuretic effect where as compound **C4** was found with the excellent diuretic activity. The research studies presented here was found to be helpful in development of new therapeutic agents with high diuretic potential.

KEYWORDS: Structural analogues, Ethacrynic acid, Diuretic potential, Molecular docking, Spectroscopy, Diuretic Index.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Ethacrynic acid is a effective loop diuretic that produces a prompt and prefound diuresis.[1] It is clinically used as diuretic drug and used to treat high blood pressure and swelling caused by diseases. Literature reveals that carboxyl group is an important pharmacophore for the diuretic effect of ethacrynic acid.[2]

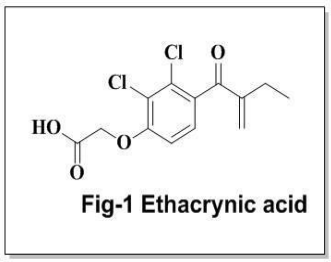


Fig-1 Ethacrynic acid

In the thick ascending loop sodium and chloride reabsorption is accomplished by Na+, K+, Cl- symporter or co-transporter (NKCC2). The thick ascending limb has a high reabsorptive capacity and is responsible for reabsorbing 25% of the filterd load of sodium. The loop diuretics act by blocking this symporter.[3] Ethacrynic acid induces diuresis primarily by inhibiting the Na+K-Cl- co-transporter (symporter) in the renal tubule that is located in the thick ascending limb of the loop of Henle. Ethacrynic acid-induced diuresis is characterized by increased sodium (up to 25% of the filtered load), potassium, chloride, calcium, magnesium, and water excretion.[4]

Na-K-ATPase is a diuretic receptor. It was found that complete inhibition of fractional sodium reabsorption occurs only at partial inhibition of Na-K-ATPase.[5]

Adenylate cyclase involved in the control of sodium transport and water permeability, ethacrynic acid also inhibits the enzyme.[5] Ethacrynic acid increases renal blood flow mediated by increase renal production of prostaglandin E.[6] Prostaglandin E2 (PGE2) inhibits sodium reabsorption within the Thick Ascending Limb (TAL) of the loop of henle and anti diuretic hormone (ADH) mediated water transport in collecting tubules.[7] Ethacrynic acid is best used in the treatment of patient with resistant oedema: in such cases it is found invaluable.[8] It is effective in all types of edema.[9] Ethacrynic acid has been found to be a potent diuretic agent in man.[10] Literature proves ethacrynic acid is an agent with high efficacy of diuretic effect. Therefore novel structural analogues were designed, docked, synthesized and diuretic activity was determined by measuring different parameters such as urine volume, urine pH, urine conductivity, urine electrolyte concentration (sodium, potassium and chloride ions).

2. MATERIALS AND METHODS

All the chemicals (reagents and solvents) used in the research study of high purity were purchased from sigma Aldrich. To assure purity and completion of reaction thin layer chromatography (TLC) was performed using pre-coated silicagel 60 F254 plates with layer thickness 0.25nm purchased from Merck ltd. Iodine vapours / U.V light were used to locate the spot on TLC chromatogram. One end open capillary tubes were used to measure the melting point on a liquid paraffin bath and are uncorrected**.** The I.R spectra were recorded in KBr by using (JASCO FT-IR 4000) spectrophotometer.^{1}HNMR and ^{13}CNMR were recorded on the Bruker Advanced (600MHz) in DMSO-d6 solution, with TMS as an internal Standard. Standard abbreviation indicating multiplicity was used as: s=singlet, d=doublet, t=triplet, q=quintet, m=multiplate. Mass spectra were recorded on high resolution liquid chromatography Mass spectrometer orbitrap (LC –MSQTOF). Purification of all the synthesized compounds was done by recrystallization followed by column chromatography using n-hexane/ethylacetate as solvent system. Statistical analysis was carried out with Graphpad Instat software.

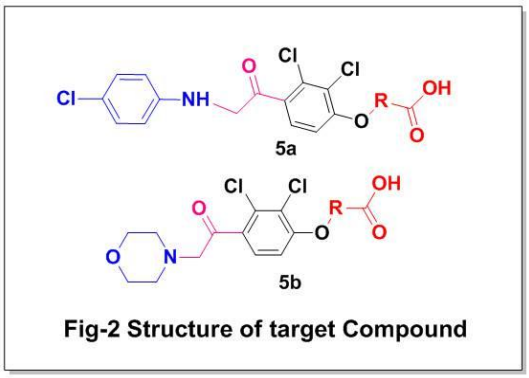


Fig-2 Structure of target Compound

3. EXPERIMENTS

3.1. Procedure for synthesis of Compound(3), (2,3-dichlorophenoxy) carboxylic acid[11]

Substituted phenol (1) (100 mmol) was added to a solution of sodium hydroxide (15 g, 375 mmol) in 30 ml of water, then 2-chlorocarboxylic acid (170 mmol) was added slowly at 40 ^{0}C. The mixture was heated to85 ^{0}C to reflux with stirring for 2 h. Two hundred milliliters of water was added after the mixture was cooled down. The solution was filtered and acidified to pH 1–2 with concentrated hydrochloric acid. The brown oil fraction was extracted twice with ether (100 mL). The ether fraction was further extracted twice with 5%sodium bicarbonate solution (75 mL). The sodium bicarbonate solution was acidified to pH 1–2 with concentrated hydrochloric acid and yielded a solid product. The solid product was collected with filtering and dried to obtain compound (3)

3.2. Procedure for synthesis of Compound (4), (2,3-dichloro-4-(2-chloroacetyl)phenoxy) Carboxylic acid[11]

The compound (3) (24 mmol) was added to carbon disulfide (70 mL) with stirring at room temperature. Powdered aluminum chloride (10 g, 75 mmol) was added into batches and then acyl chloride (30 mmol) was added slowly. The mixture was heated up to 70^{0}C to reflux for

4 h and then the carbon disulfide was decanted after cooling to the room temperature. The residue was added to a mixture of ice (100 g) and concentrated hydrochloric acid (3 ml) and yielded oil fraction. The oil fraction was extracted twice with ether (100 ml), and then the ether fraction was extracted twice with 5% sodium bicarbonate solution (50 ml). The sodium bicarbonate extract was acidified with concentrated hydrochloric acid to pH 1– 2. The solid particles were collected by filtering and dried to obtained compounds IV.

3.3. Procedure for synthesis of compound 5(5a) (2,3-dichloro-4-(2-((4-chlorophenyl)acetyl)phenoxy carboxylic acid and 5(5b) 2-(2,3-dichloro-4-(2-morpholinoacetyl)phenoxy) carboxylic acid[12]

Prepare a solution of the amine and the alkyl halide in DMF. Using an excess of either starting material is beneficial (1.5-2 equivalents). Add 1.5 equivalents of the base and, in the case the halide is a bromide or a chloride, 1.5 equivalents of sodium iodide. Stir at room temperature for 12-24h. For lower reactivity building blocks, increasing the temperature up to reflux conditions is necessary. At the end of the reaction, quench with water, extract with a suitable organic solvent (e.g. ethyl acetate) and proceed to the product purification.

3.4.1. 2-(2,3-dichloro-4-(2-((4-chlorophenyl)amino)acetyl)phenoxy)acetic acid (C1)

Yield 73% (brown solid). m.p=133-134, R_f =0.58(n-hexane:ethylacetate 3.5:1.5); ^{1}HNMR (600MHz, DMSO-d6) δ: 11.3 (s,1H), 7.8 (d, J=5.44Hz 1H), 7.4(m, 3H) 6.8(m, 2H), 4.6(s, 2H) 4.3(s,2H), 3.9(s, 1H); ^{13}CNMR (600MHz, DMSO-d6) δ: 197.7, 169.4, 158.1, 147.1, 132.0, 131.6, 130.6,130.6 129.7, 121.2, 113.1, 104.4,69.6, 58.1; LCMS (MW=388.63) m/z 388.64 (M+H)+. Calcd for M.F. =C16H12Cl3NO4; C, 49.45; H, 3.11; Cl, 27.37; N, 3.60; O, 16.47 Found C, 49.43%; H, 3.10%; Cl, 27.35%; N, 3.59%; O, 16.45% ; IR (KBr,cm^{-1})v 3505.95(NH aromatic), 3043.01 (CH aromatic), 2850.23 (C-H alkane), 2843(CH methylene) 2838.72(OH carboxyl), 1718.24(C=O), 1500.22(aromatic carbon), 600.53 (C-Cl).

3.4.2. 2-(2,3-dichloro-4-(2-morpholinoacetyl)phenoxy)acetic acid (C2)

Yield 84% (dark brown solid). m.p=171-172, R_f =0.69(n-hexane:ethylacetate 3.5:1.5);^{1}HNMR (600MHz, DMSO-d6) δ: 11.0(s, 1H), 7.65(d, J=2.16Hz,1H), 7.32(d, J=7.0Hz, 1H), 4.66 (s, 2H), 3.65-3.68 (mH, 6H)), 2.50(m, 4H); ^{13}CNMR (600MHz, DMSO-d6) δ: 195.3, 169.9, 164.3, 131.4, 121.2,110.16, 69.0, 67.0, 66.4, 66.4, 55.6,55.6; LCMS (MW=348.18) m/z 348.20 (M+H)+. Calcd for M.F. = $C_{14}H_{15}Cl_2NO_5$; C, 48.29; H, 4.34; Cl, 20.36; N, 4.02; O,22.98 Found C, 48.27%; H, 4.33%; Cl, 20.35%; N, 4.01%; O, 22.96%; IR (KBr,cm^{-1})v 3475.1(CH Morpholine), 3050.83(CH aromatic), 2992.98(C-H alkane), 3343(OHcarboxyl), 2845(CH methylene), 1670(C=O),1562.06(aromatic carbon), 650.21 (C-Cl).

3.4.3. 3-(2,3-dichloro-4-(2-((4-chlorophenyl)amino)acetyl)phenoxy)propanoic acid (C3)

Yield 70% (black solid). m.p=91-92, R_f =0.72(n-hexane:ethylacetate 3.5:1.5); ^{1}HNMR (600MHz, DMSO-d6) δ 10.9(s,1H),7.8 (d, J=2.73Hz,1H), 7.3(d, J=2.31Hz, 1H). 6.8-7.2(m, 4H), 4.66 (s, 2H), 4.3 (t, J=4.46Hz 2H), 4.1(s,1H) 2.8(t, J=4.53Hz, 2H); ^{13}CNMR (600MHz, DMSO-d6) δ: 204.4, 172.4, 159.3, 147.9,131.9, 125.9, 122.3, 111.0, 107.3, 66.1, 55.1, 38.7 LCMS (MW=402.66) m/z 464.67 (M+H)+. Calcd for M.F. = $C_{17}H_{14}Cl_3NO_4$; C, 50.71; H, 3.50; Cl, 26.41; N, 3.48; O, 15.89 Found C, 50.69%; H, 3.48%; Cl, 26.40%; N, 3.47%; O, 15.87%; IR (KBr,cm^{-1})v 3343(OHcarboxyl), 3440.95(NH aromatic), 3151.11 (C-H aromatic), 2992.98(C-H alkane), 2853(CH methylene), 1670(C=O),1562.06(aromatic carbon), 650.12 (C-Cl).

3.4.4. 3-(2,3-dichloro-4-(2-morpholinoacetyl)phenoxy)propanoic acid (C4)

Yield 65% (brown solid). m.p=114-115, R_f =0.76(n-hexane:ethylacetate 3.5:1.5); ^{1}HNMR (600MHz, DMSO-d6) δ:11.5(s, 1H), 7.8(d, J=2.46Hz,1H), 7.1(d, J=2.74Hz, 1H), 4.2 (t, J=2.19Hz, 2H), 3.3-3.6 (m, 6H)), 2.9(m, 4H), 2.6(t, J=2.12Hz, 2H); ^{13}CNMR (600MHz, DMSO-d6) δ: 197.4, 163.3, 159.3, 137.9, 128.1, 121.5,111.0, 107.3, 69.7, 63.7, 52.1, 52.1, 38.4; LCMS (MW=425.03) m/z 425.08 (M+H)+. Calcd for M.F. = $C_{15}H_{17}Cl_2NO_5$; C, 49.74; H, 4.73; Cl, 19.58; N, 3.87; O, 22.09 Found C, 49.71%; H, 4.70%; Cl, 19.59%; N, 3.85%; O, 22.04%; IR (KBr,cm^{-1})v 3575.38(CH Morpholine), 3050.60 (C-H aromatic), 2869.56(C-H alkane), 2661.28(OH carboxyl) 2852(CH methylene), 1700.91(C=O), 1508.06(aromatic carbon),705.12 (C-Cl).

3.4.5. 4-(2,3-dichloro-4-(2-((4-chlorophenyl)amino)acetyl)phenoxy)butanoic acid (C5)

Yield 77% (black solid). m.p=161-162, R_f =0.78(n-hexane:ethylacetate 3.5:1.5);^{1}HNMR (600MHz, DMSO-d6) δ: 11.6 (s,1H), 7.7 (d, J=2.73Hz, 1H), 7.4 (d, J=2.22Hz, 1H), 7-7.1(m, 4H), 4.7 (s, 2H), 4.3(t, J=1.90Hz, 2H), 4.0(s, 1H), 2.35(t, J=1.26Hz, 2H), 2.08(q, J=1.58Hz, 2H); ^{13}CNMR (600MHz, DMSO-d6) δ: 197.2, 172.8, 158.1, 147.9, 130.8, 129.7, 128.3, 128.1, 127.5, 127.2, 120.7, 115.5, 111.9, 69.3, 30.4, 23.0; LCMS (MW=416.68) m/z 416.70 (M+H)+. Calcd for M.F. = $C_{18}H_{16}Cl_3NO_4$; C, 51.88; H, 3.87; Cl, 25.53; N, 3.36; O, 15.36 Found C, 51.85%; H, 3.88%; Cl, 25.51%; N, 3.34%; O, 15.33%; IR (KBr,cm^{-1})v 3478.95(NH aromatic), 3050.60 (C-H aromatic), 2869.56(C-H alkane), 2661.28(OH carboxyl), 2847(CH methylene) 1700.91(C=O), 1508.06(aromatic carbon),705.15 (C-Cl).

3.4.6. 4-(2,3-dichloro-4-(2-morpholinoacetyl)phenoxy)butanoic acid (C6)

Yield 80% (red solid). m.p=145-146, R_f =0.61(n-hexane:ethylacetate 3.5:1.5);^{1}HNMR (600MHz, DMSO-d6) δ: 10.9(s, 1H), 7.7(d, J=2.48Hz,1H), 7.32(d, J=2.66Hz, 1H), 4.0(t, J=2.73Hz, 2H), 3.5-3.68 (mH,

6H)), 2.9(m, 4H), 2.6(t, J=2.06Hz, 2H), 2.08(q, J=2.90Hz, 2H); ^{13}CNMR (600MHz, DMSO-d6) δ: 197.3, 172.8, 158.1, 137.9, 130.8, 128.3, 128.1, 120.7, 110.2, 79.4, 69.3, 66.1, 52.8, 52.8, 30.47, 23.0 ; LCMS (MW=376.23) m/z 376.25 (M+H)+. Calcd for M.F. = $C_{16}H_{19}Cl_2NO_5$; C, 51.08; H, 5.09; Cl, 18.85; N, 3.72; O, 21.26 Found C, 51.05%; H, 5.06%; Cl, 18.82%; N, 3.75%; O, 21.29%; IR (KBr,cm^{-1})v 3475.16(CH Morpholine), 3100.97 (C-H aromatic), 2919.7(C-Halkane), 3343.96(OHcarboxyl),2860(CH methylene), 1739.48(C=O),1515.78(aromatic carbon), 755.95 (C-Cl).

3.4.7. 3-(2,3-dichloro-4-(2-((4-chlorophenyl)amino)acetyl)phenoxy)-2,2-dimethylpropanoic acid(C7)

Yield 69% (black solid). m.p=104-105, R_f =0.79(n-hexane:ethylacetate 3.5:1.5) ^{1}HNMR (600MHz, DMSO-d6) δ: 11.0 (s,1H),7.65(d, J=2.4Hz, 1H), 7.32(d, J=7.1Hz, 1H), 6.54-7.27(m, 4H), 4.55(s, 2H), 4.13(s, 2H), 4.0(s, 1H), 1.28(s, 6H); ^{13}CNMR (600MHz, DMSO-d6) δ: 195.3, 179.0, 157.2, 145.7, 131.4, 129.6, 129.6, 126.1, 121.3, 114.9, 114.9, 110.2, 79.4, 59.9, 43.3, 21.2, 21.2; LCMS (MW=430.71) m/z 430.70 (M+H)+. Calcd for M.F. = $C_{19}H_{18}Cl_3NO_4$; C, 52.98; H, 4.21; Cl, 24.69; N, 3.25; O, 14.86 Found C, 52.96%; H, 4.20%; Cl, 24.67%; N, 3.22; O, 14.83%; IR (KBr,cm^{-1})v 3317.8(NH aromatic), 3147.26 (C-H aromatic), 2850.32 (C-Halkane), 3301.54(OH carboxyl), 2849(CH methylene), 1762.(C=O),1550.49(aromatic carbon), 1411.35 (CH_2),710(C-Cl).

3.4.8. 3-(2,3-dichloro-4-(2-morpholinoacetyl)phenoxy)-2,2-dimethylpropanoic acid (C8)

Yield 84% (black solid). m.p=124-125, R_f =0.67(n-hexane:ethylacetate 3.5:1.5); ^{1}HNMR (600MHz, DMSO-d6) δ: 11.0(s, 1H), 7.65(d, J=2.16,1Hz, 1H), 7.32(d, J=7.0Hz, 1H), 4.13(s,2H), 3.65-3.68(m,6H), 2.50(m, 4H), 1.28(s,6H)^{13}CNMR (600MHz, DMSO-d6) δ: 195.3, 179.0, 131.4, 131.0, 128.9, 121.3, 110.2, 79.4, 69.9, 66.4, 66.4, 57.2, 55.6, 55.6, 43.0, 21.2, 21.2; LCMS (MW=390.26) m/z 390.28 (M+H)+. Calcd for M.F. = $C_{17}H_{21}Cl_2NO_5$; C, 52.32; H, 5.42; Cl, 18.17; N, 3.59; O, 20.50 Found C, 52.29%; H, 5.40%; Cl, 18.14%; N, 3.57; O, 20.51%; IR (KBr,cm^{-1})v 3397.96(CH Morpholine), 3000.7 (C-H aromatic), 2895.2 (C-Halkane), 3301.5(OH carboxyl), 2850(CH methylene), 1689.34(C=O), 1550.49(aromatic carbon), 1422.35 (CH3), 720 (C-Cl).

3.4.9. 5-(2,3-dichloro-4-(2-((4-chlorophenyl)amino)acetyl)phenoxy)pentanoic acid(C9)

Yield 72% (black solid). m.p=150-151, R_f =0.74(n-hexane:ethylacetate 3.5:1.5); ^{1}HNMR (600MHz, DMSO-d6) δ 11.0 (s,1H), 7.65(d, J=2.8Hz,1H) 7.32 (d, J=7.19Hz, 1H) 6.54-7.27 (m, 4H), 4.55 (s, 2H), 4.06(t, J=1.7Hz, 2H), 4.0(s, 1H), 2.30(t, J=1.34Hz, 2H),1.76(q, J=1.4Hz, 2H), 1.52(q, J=1.49Hz, 2H); ^{13}CNMR (600MHz, DMSO-d6) δ, 195.3, 178.4, 145.7, 131.4, 131.0, 129.6, 129.6, 129.6, 129.7, 128.9, 126.1, 121.3, 114.9, 114.9, 110.2, 67.9, 59.9, 57.2, 34.0, 28.0, 21.0; LCMS (MW=430.71) m/z 430.90 (M+H)+. Calcd for M.F. = $C_{19}H_{18}Cl_3NO_4$; C, 52.98; H, 4.21; Cl, 24.69; N, 3.25; O, 14.86 Found C, 52.95%; H, 4.19%; Cl, 24.70%; N, 3.24; O, 14.87%; IR (KBr,cm^{-1})v 3509.51(NH aromatic), 3000.7 (C-H aromatic), 2895.2 (C-Halkane), 2600.52(OH carboxyl), 2900(CH methylene) 1689.34(C=O), 1550.49(aromatic carbon), 1422.35 (CH3),720 (C-Cl).

3.4.10. 5-(2,3-dichloro-4-(2-morpholinoacetyl)phenoxy)pentanoic acid(C10)

Yield 65% (cream solid). m.p=147-148, R_f =0.76(n-hexane:ethylacetate 3.5:1.5)^{1}HNMR (600MHz, DMSO-d6) δ:11.0(s, 1H), 7.65(d, J=2.16Hz, 1H), 7.32(d, J=7.0Hz, 1H), 4.06 (t, J=1.8Hz, 2H), 3.65-3.68 (m, 6H)), 2.50(m, 4H), 2.30(t, J=1.34Hz, 2H), 1.76(q, J=1.4Hz, 2H), 1.52(q, J=1.49Hz, 2H); ^{13}CNMR (600MHz, DMSO-d6) δ:195.3, 178.4, 157.2, 131.4, 131.0, 128.9, 121.3, 110.2, 69.9, 67.9, 66.4, 66.4, 55.6, 55.6, 34.0, 28.0, 21.0; LCMS (MW=390.26) m/z 390.28 (M+H)+. Calcd for M.F. = $C_{17}H_{21}Cl_2NO_5$; C, 52.32; H, 5.42; Cl, 18.17; N, 3.59; O, 20.50FoundC,52.30%;H,5.39%;Cl,18.15%;N,3.57;O,20.47%;IR(KBr,cm^1)v3475.1(CHMorpholine),3062.41(CHaromatic),2946.71(CHalkane),3367.1(OHcarboxyl),2855(CHmethylene)1685.48(C=O),1550.49(aromatic carbon), 1465.3 (CH2), 717 (C-Cl).

3.5. BIOLOGICAL EVALUATION

3.5.1. Acute toxicity study[13]

Adverse effect of bioactive compounds can be determined by toxicological studies. Acute toxicity studies were carried out prior to the conductance of the actual experiment. It is performed with five groups of rats which orally received 50mg/kg of dose of synthesized compounds. Animals were observed for the adverse effects for the first five hours during 24 hours period and then followed for 14 days for any mortality. Novel synthesized compounds did not produce any visible signs of adverse effects which was evidenced by no change in behavioral pattern, absence of tremor, loss of body weight, convulsion, salivation, diarrhea, sleep, lethargy, paralysis, coma and mortality, suggesting the LD_{50} is greater than 50mg/kg.

3.5.2. Diuretic activity[14]

Diuretic activity was measured in Albino Wistar rats. Sixty healthy Albino Wistar rats of either sex weighing between 100-180g were obtained and acclimatized for one week prior to the experiment, all the rats were deprived of water but not food for 18 hrs. Their urinary bladders were emptied by gentle compression of the pelvic area and by pull of their tails. Each of these rats was then orally administered with 5 ml/100gm body weight of isotonic saline (NaCl, 0.9% w/v) to impose a uniform water load. Forty-five minutes later, these rats were randomly assigned into twelve groups (N = 5 per group) comprising of five animals in each group. Group-I served as normal control and received normal saline,

group II were pretreated with ethacrynic acid 10 mg/kg, (standard drug) group III-XII, treated with synthesized compounds 50mg/kg treated orally. Each of these rats was individually placed in metabolic cages which was fitted with stainless steel sieves to retain and separate feces and allow the urine to pass alone, a cumulative urine output was determined at 2 hrs intervals for 8 hrs. The color of urine was also noted. Diuretic activity is determined by measuring cumulative urine excretion volume in ml, pH by pH meter, conductivity by conductometer, Na^+ and K^+ levels by flame photometry, Na^+/K^+ ratio and Diuretic Index/diuretic action was determined. The Diuretic Index was calculated using following formula

Diuretic Index/Diuretic action=Urine volume of test group/ urine volume of control group.

3.6. COMPUTATIONAL METHODS

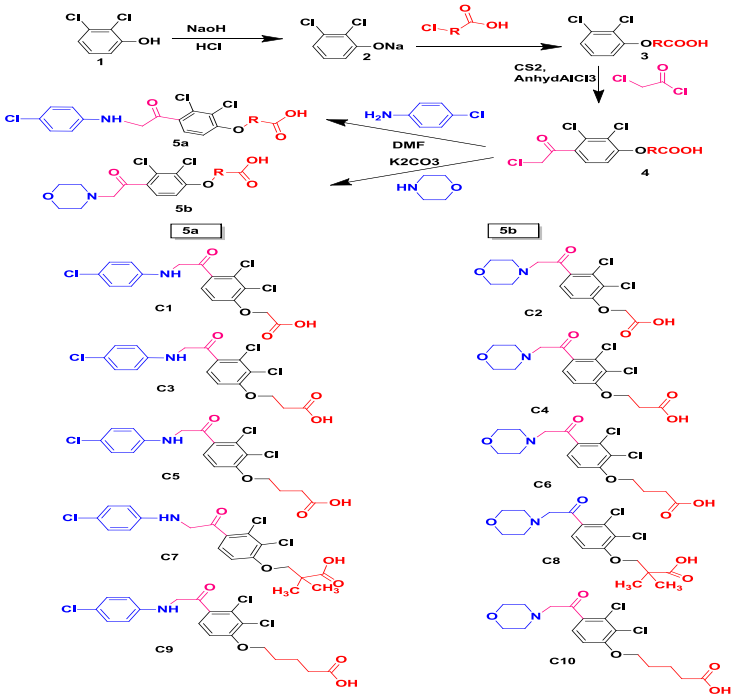
3.6.1. Molecular Docking[15]: The Glide (Schrodinger 11.8) software was used to dock potential inhibitors (Ligand) in the binding pocket of the enzyme structure. Docking studies were carried out against NKCC2 (PDB: 5DBX, https://www.rcsb.org/structure/5DBX). Receptor Grid Generation panel which define receptor structure by excluding any co-crystallized ligand that may be present, determine the position and size of the active site as it will be represented by receptor grids and set up Glide constraints. Ligand preparation was carried out using LigPrep panel in the software. All the structures in. mol format were imported in the project file and subjected to ligand preparation using OPLS 2005 force field using default setting. The ligands were docked with the active site using the 'Extra precision' Glide algorithm.

3.6.2. ADME prediction[16]: All the analogues were subjected in Qikprop for ADME prediction. It predicts both physicochemical and pharmacokinetic relevant properties. It also evaluates the acceptability of analogues based on Lipinski's rule of 5 which is essential to ensure drug like pharmacokinetic profile.

4. RESULT

4.1. Chemistry

In the research project the synthesis of novel structural analogues of ethacrynic acid. 2,3-dichloro-4-(2-aminoacetyl)phenoxy)carboxylic acid was achieved. The structure of synthesized compounds was confirmed by their spectral characterization I.R, NMR and mass spectrometry. The finished target compounds obtained in the yield of (67-88%). Purification of all the synthesized compounds were done by column chromatography using n-hexane /ethylacetate as solvent system. The target compounds were obtained by synthetic route given in scheme-1. Compound (2) is obtained by reaction of 2,3-dichlorophenol with chloro substituted carboxylic acids in presence of sodium hydroxide. Compound (2) on acidification with concentrated hydrochloric acid yielded compound (3). Compound (3) on friedel-craft acylation reaction with chloracetylchloride in presence of anhydrous aluminum chloride in carbon disulphide solvent resulted formation of compound (4). Compound (4) on amination in dimethyl formamide solvent in presence of potassium carbonate resulted in the formation of target compound (5). Total 10 derivatives of fifth compound were synthesized. In the fiftth compound R belongs to five different type of carboxylic acids. Each fourth Intermediate compound containing five different carboxylic acids on reaction with Para-chloro aniline and Morpholine generated ten novel structural analogues of ethacrynic acid.



SCHEME

Reagents and conditions : Synthesis of target compounds **(5a, 5b)**Reagents and conditions :1) synthesis of Comp **(3), (2,3-dichlorophenoxy) carboxylic acid**, chlorocarboxylic acid, NaOH, reflux with stirring,85^{0}C, 2h, 2)synthesis of compound **(4) (2,3-dichloro-4-(2-chloroacetyl)phenoxy)carboxylic acid,** chloroacetyl chloride, CS_2, anh AlCl3 reflux, 70^{0}C, 4h 3) synthesis of comp **(5)-5a (2,3-dichloro-4-(2-((4-chlorophenyl)amino)acetyl)phenoxy carboxylic acid, (5)-5b, 2-(2,3-dichloro-4-(2-morpholinoacetyl)phenoxy)carboxylic acid** amines (Parachloroaniline, Morpholine) DMF, KOH, stirring, refluxing 95^{0}C, 24 hrs.

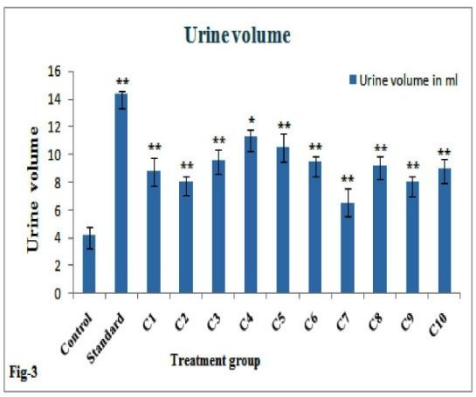
4.2. Diuretic activity

Statistical analysis: Data are expressed as mean ± SEM (Standard error of mean) statistical analysis of the data were performed with one-way analysis of variance (ANOVA) followed by Dunnets comparison test with standard EtA (Ethacrynic acid).Urine output and urinary electrolyte excretion was found to be higher and comparable with standard drug EtA(Ethacrynic acid). Urine Ph was found acidic to alkaline and urine conductivity was found to be increased.

Table 1: Effect of ethacrynic acid analogues on Urine volume and Diuretic action/Index.

Compound name	Urine volume in ml	Diuretic action/ Diuretic Index
Control	4.22± 0.529	-
Standard	14.34± 0.278**	3.44
C1	8.78± 1.02**	2.10
C2	8.06± 0.416**	1.92
C3	9.58± 0.781**	2.29
C4	11.28± 0.546*	2.69
C5	10.52± 0.960**	2.51
C6	9.480± 0.44**	2.26
C7	6.560± 1.030**	1.56
C8	9.22± 0.693**	2.20
C9	8.00± 0.419**	1.91
C10	8.98± 0.700**	2.14

All the data are expressed mean ± SEM (standard error of mean). *p<0.05, **p<0.01 when compared with standard. Diuretic action=Urine volume of test group/ urine volume of control group.



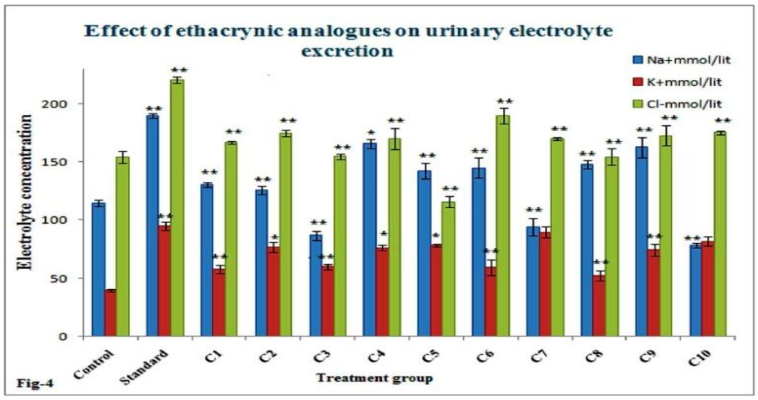


Table 2. Effect of ethacrynic analogues on urinary electrolyte excretion.

Compound name	Na^+mmol/lit	K^+mmol/lit	Cl^-mmol/lit
Control	114.80 ± 2.70	39.60± 1.20	154.20± 5.23
Standard	189.60 ± 2.06**	94.80± 3.55**	220.80±2.51**
C1	130.80 ± 1.93**	57.80± 3.80**	167.20±1.15**
C2	125.80± 3.61**	76.80± 4.25*	174.60±2.90**
C3	86..80 ± 4.16**	60.00± 2.34**	155.00±2.38**
C4	165.80 ± 3.89*	76.400± 2.31*	170.00±9.14**
C5	142.60 ± 6.87**	78.48± 1.21*	115.80±4.81**
C6	145.20 ± 8.57**	59.18±6.77**	189.80±6.82**
C7	94.40 ± 7.46**	89.80± 4.48	170.00±1.22**
C8	148.00 ± 3.67**	52.20± 4.27**	154.60±6.87**
C9	162.60± 8.98**	74.40± 4.95**	172.80±8.47**
C10	78.20± 2.01**	81.60± 3.97	175.40±1.53**

All the data are expressed mean ± SEM (standard error of mean), **p<0.01 when compared

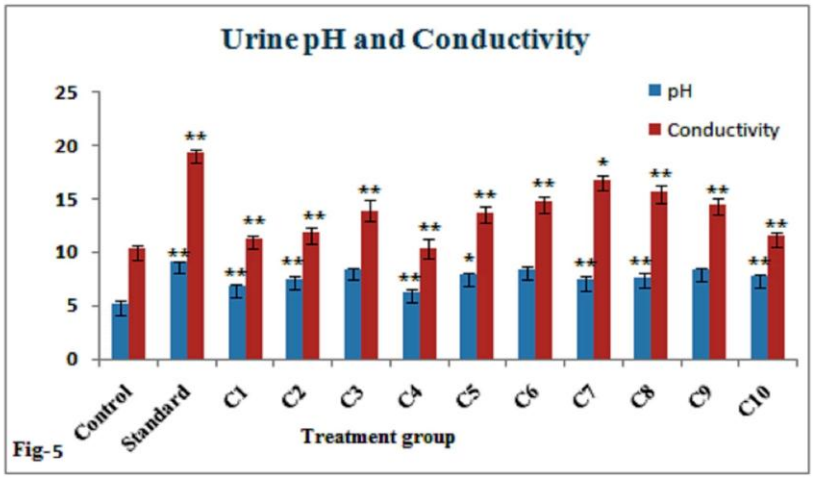


Fig-5

Table 3: Effectof ethacrynic analogues on urinary pH and Conductivity

Compound name	pH	Conductivity
Control	5.17± 0.39	10.36± 0.37
Standard	9.08±0.06**	19.39±0.25**
C1	6.87±0.17**	11.29±0.34**
C2	7.51±0.20**	11.87±0.51**
C3	8.43±0.07	13.93±0.98**
C4	6.32±0.17**	10.39±0.92**
C5	7.86±0.20*	13.72±0.60**
C6	8.46±0.21	14.75±0.45**
C7	7.41±0.34**	16.83±0.36*
C8	7.66±0.47**	15.68±0.67**
C9	8.31±0.15	14.49±0.57**
C10	7.77±0.20**	11.55±0.34**

All the data are expressed mean ± SEM (standard error of mean), *p<0.05, **p<0.01 when compared with standard.

4.3. Molecular Docking

Molecular docking study was done to determine favorable accommodation of novel molecules in binding pocket of the receptor. All the compound were docked against NKCC2 (PDB: 5DBX) using Ethacrynic acid as standard**.** The docking results indicates that all the designed compounds have maximum score as compare to standard drug (Table 4). Ligand interaction as shown in Figure-6 which gives an idea of H-bond interactions with the residues LYS104, ALA85, LEU153, MG402, GLY205 and ARG13.

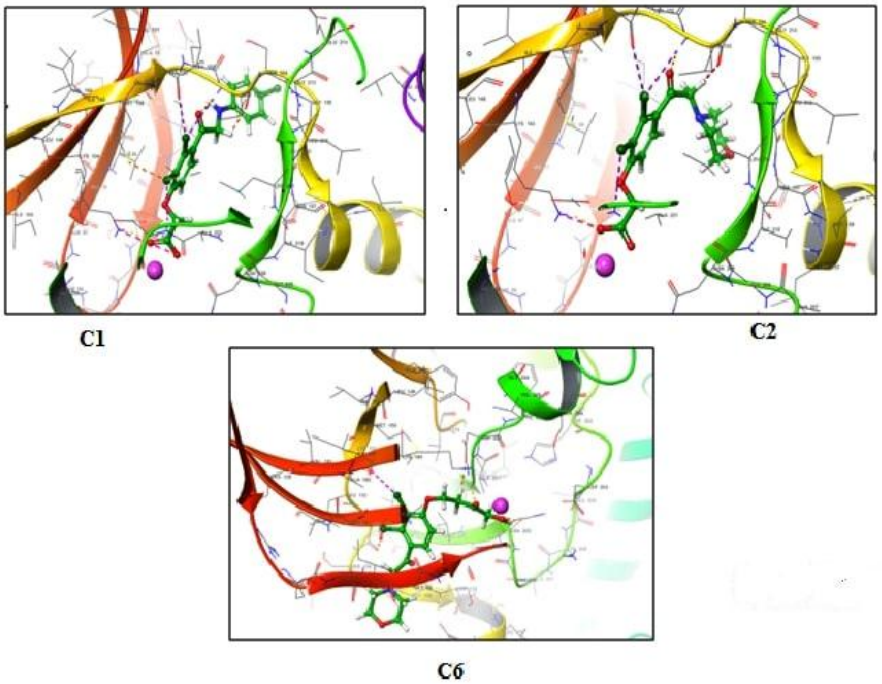


Fig-6 Best docking poses of compound C1, C2, C6

Table 4. Docking interaction of compound with NKCC2.

Compound	Dock score
C1	-10.584
C2	-10.235
C3	-9.837
C4	-10.116
C5	-9.622
C6	-10.714
C7	-10.601
C8	-6.741
C9	-8.619
C10	-9.236
EtA(Std)	-9.106

4.4. ADME Studies: The parameters illustrated in (Table-5) Qikprop analysis show significant results. CNS parameter is related with absorption of entity through Blood brain barrier, standard limit for CNS is -2 to +2, where -2 shows inactive CNS penetration indicate compound with no CNS toxicity and +2 shows active CNS penetration indicate designed compound having CNS toxicity. All the designed entities show satisfactory results within the range. Here all entities shows more than 80% oral absorption, it is considered to be highly absorbed. Number of hydrogen bond donner, number of hydrogen bond acceptor and number of rotatable bond count as well as partition coefficient of all designed NCEs are in range. Therefore, ADME results indicates that designed derivatives possess drug like pharmacokinetic properties. Based on result of docking and ADME prediction the compounds follow all screening criteria were selected for synthesis and biological evaluation to verify the results of In Silico Study.

Table. 5 Physicochemical and Pharmacokinetic studies.

Compound Name	MW	LogP	PSA	%ABS	CNS	HBD	HBA	Toxicity
C1	388.634	3.772	97.127	81.639	-2	2	5.75	Non toxic
C2	348.182	-0.459	94.179	53.435	0	1	8.45	Non toxic
C3	402.661	4.228	94.938	85.677	-2	2	5.75	Non toxic
C4	362.209	-0.016	94.900	54.395	0	1	8.45	Non toxic
C5	416.688	4.641	92.956	89.906	-2	2	5.75	Non toxic
C6	376.236	0.923	94.001	70.216	1	1	10.15	Non toxic
C7	430.714	4.987	87.984	95.013	-1	2	5.75	Non toxic
C8	390.263	0.708	89.590	61.681	0	1	8.45	Non toxic
C9	430.714	4.954	94.923	89.872	2	2	5.75	Non toxic
C10	390.263	0.686	96.117	56.881	-1	1	8.45	Non toxic
EtA(Std)	303.141	2.995	82.269	81.018	-1	1	4.75	Non toxic

MW, molecular weight; LogP, logarithm of partition coefficient of compound between n-octanol and water; PSA, polar surface area; %ABS, percent of human oral absorption; CNS, absorption through blood brain barrier; HBA, hydrogen bond donor; and HBD, hydrogen bond acceptors;

5. DISCUSSION

Structure-Activity Relationship (SAR)

All the compounds possess Methylene group, acdjacent ketone group and two chlorine atoms on second and third position of aromatic ring of phenoxy carboxylic acid significant for the diuretic activity. Results of biological evaluation suggest that diuretic activity increases with the newly synthesized compounds. Compound **C3** is the phenoxypropanoic acid possessing chlorophenylaminoacetyl group and compound **C5** also having chlorophenylaminoacetyl group which is a phenoxybutanoic acid are the good diuretic agents where as compound **C4** is a phenoxybutanoic acid possessing morpholinoacetyl group was found with the highest diuretic potential in the series.

CONCLUSION:

Novel structural analogues of ethacrynic acid were synthesized. Spectral techniques were used to confirm the structure of synthesized compound. Binding mode and affinity of the novel compounds were determined by molecular docking studies. ADME prediction was done by Qikprop analysis. The diuretic activity was meseaured by using experimental animal model (Albino Wistar rats). The compound **C3, C4, C5, C6** were found to have increased diuretic activity.

These research studies may provide valuable insight for further modification in increasing therapeutic efficacy.

LIST OF ABBREVIATIONS: NKCC2, Sodium potassium Chloride Co-transporter; PDB- Protein data bank; EtA, Ethacrynic acid; ANOVA, Analysis of variance; NCEs, New Chemical Entities; ADME, absorption, distribution, metabolism, excretion.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The research study was carried out with the use of animals (Albino wistar rats). Experimental protocol for the biological activity (diuretic activity) was approved by Institutional Animal Ethics committee CPCSEA/IAEC/P'cology-67/2019-20/166 dated13th January 2020.procedures followed in accordance with the standards set forth in the eighth edition of guide for the care and use of laboratory animals.

HUMAN AND ANIMAL RIGHTS
Research work on animals was carried out in accordance with the NC3Rs ARRIVE guidelines.

CONSENT FOR PUBLICATION
Not applicable

AVAILABILITY OF DATA AND MATERIALS
All the data have been made available in the " Supplementary material" section

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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AUTHORS CONTRIBUTIONS
The idea or hypothesis was construted and resarch study was designed by Dr.SNM. Planning the methods to generate hypothesis, or to reach the conclusion was performed by Dr.SAA, Dr. SNM, Ms. AB. Provision of personnel, environmental and financial support and equipment and instruments that are vital for the project was made by Ms. AB and Dr. SNM. Novel compound synthesis, purification, data collection was done by Ms. AB. Data analysis and interpretation was done by Ms.SM. Biological screening, Mnuscript writing and revising was done by Dr.SAA and Ms. AB.

DATA AVAILABILITY STATEMENT
All the data have been made available in the " Supplementary material" section

ABBREVIATIONS: NKCC2, Sodium potassium Chloride Co-transporter; PDB- Protein data bank; EtA, Ethacrynic acid; ANOVA, Analysis of variance; NCEs, New Chemical Entities; ADME, absorption, distribution, metabolism, excretion.

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