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Design and synthesis of some novel 6-methoxynaphthalene derivatives with Potential anticancer activity

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ABSTRACT

Starting from the naphthalene derivative, which belongs to the arylpropanoic acid family of NSAIDs, certain 6-methoxynaphthalene derivatives have been synthesized, and evaluated for their antiproliferative activities. The preliminary assays indicated that compounds ***6b-d, 16*** *were found to have promising inhibitory activity against Colon cancer cell line HCT-116. Molecular modeling and docking of the active compounds into AKR1C3 complexed with its bound inhibitor Indomethacin using Molsoft ICM 3.4-8C program was performed in order to predict the affinity and orientation of the synthesized compounds at the active site. Detailed synthesis, spectroscopic and biological data are reported.*

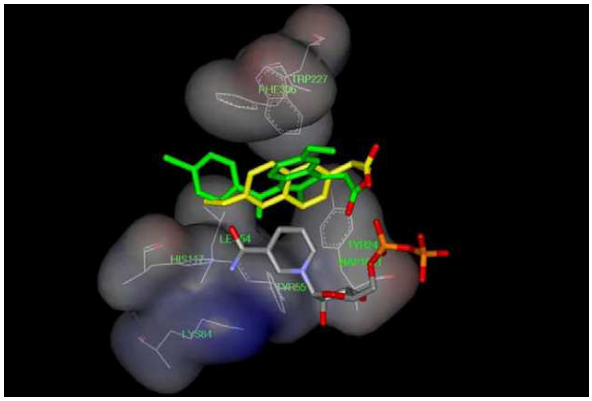
Keywords: 6-Methoxynapthalenes/anticancer/ AKR1C3/docking studies.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used to control inflammatory diseases by inhibiting COX and, in particular, COX-2 activity. It was well established that NSAIDs protect against tumors of the gastrointestinal tract and a variety of other tumors. Moreover, epidemiological studies provide evidence that the patient chronically consuming NSAIDs is associated with reduced risk of breast, prostatic and colon cancer [1-3]. Recently, Desmond et al. suggested that NSAIDs could exert their anti-neoplastic activities via a non-COX-2 pathway [4]. AKR1C3, is a member of NAD(P)H-dependent aldo-Keto reductase superfamily which has a broad tissue distribution, and which provides a potential mechanism for COX-independent anticancer effect of NSAIDs [5,6]. A selective inhibitor of AKR1C3 would be an important tool for understanding its role in cancer development [7].

Nonsteroidal anti-inflammatory drugs (NSAIDs) like indomethacin, flufenamic acid, diclofenac and naproxen have been recently identified as potent inhibitors of AKR1C3 with the IC50 values in the low micromolar range. AKR1C3 converts PGD2 into PGF2a, thereby preventing its conversion to 15-Δ12,14-PGJ2, a natural ligand for the peroxisome proliferator-activated receptor-c(PPARγ). Inhibitors of AKR1C3 are thus potential anti-neoplastic agents as they can indirectly activate PPARγ receptor by diverting PGD2 catabolism to the generation of J-series prostanoids. Activation of PPARγ receptor induces differentiation and causes apoptosis in many cell types and cancers [4, 5]. Although AKR1C3 is a promising therapeutic target, only a few inhibitors have been reported so far [8]. Development of inhibitors that consist of nonsteroid core, and are thus devoid of residual steroidogenic activity, would be especially attractive.

Indomethacin, which is considered a potent inhibitor of the AKR1C3, NADP+ indomethacin complex demonstrates coordination between indomethacin's bridge carbonyl group and the catalytic Tyr55. This interaction position indomethacin in the AKR1C active site in a way favours the binding to AKR1C3. Naproxen was docked into the AKR1C3 active site, it occupied a similar position of active site as indomethacin. It binds to the bottom of the active sites' hydrophobic pocket and on top of the coenzymes' nicotinamide moiety. Also, naproxen's carboxylate could form H-bonds with the oxygen atoms of the NADP+ diphosphate moiety. This binding mode suggests that it was as an even better inhibitor [5, 9].



Superimposition of the computer model of naproxen (in yellow, carboxylate oxygens in red) on the X-ray structure of indomethacin (in green, carboxylate oxygens in red) bound toAKR1C3

Design of drug targets containing two carboxylic groups appropriately attached to the opposite sides of the aromatic fragment (e.g., naphthalene ring) as shown by our synthesized compounds to interact with the active site hydrophobic pocket while the first carboxylate may occupy the oxyanion hole and the second forms H-bonds with oxygen of coenzyme's diphosphate moiety will be a good rational for potent inhibitors.

MATERIALS AND METHODS

Elemental analysis (C, H, N) were performed on Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA) at the Microanalytical Unit of Cairo university. All melting points were measured in open capillary tubes using Griffen apparatus and are uncorrected. Progress of the reaction was monitored by TLC using TLC sheets precoated with UV fluorescent silica gel Merck 60 F254 plate and were visualized using UV lamp. The ^{1}H-NMR spectra were recorded on Varian Gemini EM-300 MHz. NMR spectrophotometer at Research Services Unit, Faculty of Science, Cairo University. DMSO-d_6 was used as a solvent, the chemical shifts were measured in ppm, relative to TMS as an internal standard.

Chemistry

Compounds (2, 3, 5, 7, 8) were prepared according to the reported methods.[10-13]

5-(1-(6-Methoxynaphthalen-2-yl)ethyl)-1,3,4-thiadiazol-2-amine (4)

A mixture of compound **(3)** (0.01 mol, 3 g) and phosphorus oxychloride (10 ml) were refluxed together at 75 ^{o}C for 5 h. The reaction mixture was allowed to cool then poured dropwise on to ice-cold water, the solid product so formed was filtered, dried and crystallized from ethanol.

Yield (1.5 g, 53.5%); **m.p**. (217-220 ^{o}C), **Analysis**% for $C_{15}H_{15}N_3OS$ (285.36) Calcd. (Found.) C: 63.13 (63.60), H: 5.30 (5.23), N: 14.73 (14.29). **IR** (cm^{-1}): 3190 (NH_2), 1616 (C=N), **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.42-1.45 (m, 3H, CH_3), 3.69-3.72 (q, 1H. C<u>H</u>CH_3), 3.85 (s, 3H, OCH_3), 7.12- 7.48 (m, 3H, ArH), 7.71- 7.87 (m, 3H, ArH), 10.05 (d, 2H, NH_2, D_2O exchangeable). **MS** (m/z): 285 (M^+, 8.97%), 270 (5.9%), 243(19.9 %), 185 (100%).

4-(3-(1-(6-Methoxynaphthalen-2-yl)ethyl)ureido)-N-(substituted)benzenesulfonamide (6a-d)

A mixture of equimolar amounts (0.01 mol, 2.5 g) of compound (**5)** and the appropriate sulphonamides (0.01 mol) in DMF (10 ml) containing few drops of pyridine was refluxed for 3 h. The reaction mixture was allowed to cool then poured on to ice-cold water acidified with HCl, the solid so obtained was filtered, dried and recrystallized from ethanol.

6a: (R=H), *4-(3-(1-(6-methoxynaphthalen-2-yl)ethyl) ureido) benzenesulfonamide*
Yield (1.2 g, 76%); **m.p**. (165-168) ^{o}C, **Analysis** % for $C_{20}H_{21}N_3O_4S$ (399.46) Calcd. (Found.) C: 60.13 (60.81), H: 5.30 (5.21), N: 10.52 (9.74). **IR** (cm^{-1}): 3418, 3200 (NH), 1620 (CO), **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.42-1.46 (m, 3H, CH_3), , 3.85 (s, 3H, OCH_3), 4.87-4.85 (q, 1H. C<u>H</u>CH_3, J=6.6 Hz), 6.40 (br s, 1H. NH, D_2O exchangeable), 6.66- 7.77, (m, 11H, ArH), 8.60, (s, 2H, NH_2, D_2O exchangeable) 9.40 (s, 1H. NH, D_2O exchangeable), **MS** (m/z): 399 (M^+,7.75%), 377 (5.90%), 214 (30.30 %), 186 (100%).

6b: (R=$COCH_3$), *N-(4-(3-(1-(6-methoxynaphthalen-2-yl)ethyl)ureido)phenyl sulfonyl)acetamide*,
Yield (1.6 g, 89%); **m.p**. (167-170^{o}C), **Analysis** % for $C_{22}H_{23}N_3O_5S$ (441.50) Calcd. (Found.) C: 59.85 (59.90), H: 5.25 (5.43), N: 9.52 (9.10). **IR** (cm^{-1}): 3350, (NH), 2964(CH-aliphatic) 1622 (CO), **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.37 (d, 3H, CH_3, J=6.9 Hz), 1.90 (s, 3H, $COCH_3$) , 3.84 (s, 3H, OCH_3) 4.86 (q, 1H. C<u>H</u>CH_3, J=6.9), 7.11- 8.90 (m, 13H, ArH+ 3NH D_2O exchangeable), **MS** (m/z): 445 (M+4, 4.85%), 214 (30.30 %), 185 (20.79%), 76 (100%).

6c: (R=2-pyridine), *4-(3-(1-(6-methoxynaphthalen-2-yl)ethyl)ureido)-N-(pyridin-2-yl)benzenesulfonamide*
Yield (0.75 g, 39%); **m.p**. (170-173^{o}C), **Analysis** % for $C_{25}H_{24}N_4O_4S$ (476.55) Calcd. (Found.) C: 63.01 (63.33), H: 5.08 (5.40), N: 11.76 (11.95). **IR** (cm^{-1}): 3312 (NH), 2934 (CH-aliphatic), 1624 (CO), **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.35-1.37, (d, 3H, CH_3), 3.85 (s, 3H, OCH_3), 4.83 (q, 1H, C<u>H</u>CH_3), 6.46 (s, 1H, NH D_2O exchangeable), 7.10-8.83 (m, 16H, ArH, 2NH D_2O exchangeable). **MS** (m/z): 476 (M^+, 0.85%), 397 (3.82%), 211(15.78%), 185(100%).

6d: (R=2-thiazole), *4-(3-(1-(6-methoxynaphthalen-2-yl)ethyl)ureido)-N-(thiazol-2-yl)benzenesulfonamide*
Yield (0.9 g, 46%); **m.p**. (241-244^{o}C), **Analysis** % for $C_{23}H_{22}N_4O_4S$ (482.58) Calcd. (Found.) C: 57.24 (57.21), H: 4.60 (4.82), N: 11.61 (11.49). **IR** (cm^{-1}): 3346 (NH), 2964 (CH-aliphatic) 1620 (CO), 1566 (CN), **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.36-1.41 (m, 3H, CH_3,), 3.87 (s, 3H, OCH_3), 4.85-4.87 (q, 1H. C<u>H</u>CH_3), 6.37-6.39 (d, 1H, ArH J=7.2 Hz), 6.39-8.72 (m, 14H, ArH, 3NH D_2O exchangeable) **MS** (m/z): 482 (M^+,1.95%), 428 (11.90 %), 243 (18.10%), 200(100%).

N-Cyclohexyl-2-(2-(6-methoxynaphthalen-2-yl)propanoyl)hydrazinecarbothioamide (9)
A mixture of equimolar amounts (0.01 mol, 2.4 g) of naproxen acid hydrazide (**8**) and cyclohexyl isothiocyanate (0.01 mol, 1.4 g) in dioxane (20 ml) was refluxed for 8 h on a water bath. The reaction mixture was then concentrated, cooled, and kept overnight in the refrigerator. The solid separated out was filtered, dried, and crystallized from ethanol.

Yield (1.8 g, 47.4%); **m.p**. (200-203 ^{o}C), **Analysis** % for $C_{21}H_{27}N_3O_2S$ (385.52) Calcd. (Found.) C: 65.42 (65.45), H: 7.06 (5.99), N: 10.90 (11.29). **IR** (cm^{-1}): 3372, 3240 (3NH), 2930-2854 (CH-aliphatic), 1670 (CO), 1176 (CS). **^{1}HNMR** (DMSO-d_6) δ (ppm) : 0.72 (br, 3H, Cyclohexyl H), 1.04 (br, 4H, Cyclohexyl H), 1.52 (br, 4H, Cyclohexyl H), 1.44-146 (d, 3H, CH_3, J=6.9 Hz), 3.56 (s, 1H, NH D_2O exchangeable), 3.78 (m, 1H, C<u>H</u>CH3), 3.86 (s, 3H, OCH_3), 7.13-7.17, (d, 1H, ArH_e, J=8.7 Hz), 7.29 (s, 1H, ArH_d), 7.46-7.49 (d, 1H, ArH_b), 7.64-7.78, (m, 3H, $ArH_{a,c,f}$+1H, SH), 9.17 (s, 1H, CSN<u>H</u>, D_2O exchangeable), 9.94 (s, 1H, CON<u>H</u> D_2O exchangeable), **MS** (m/z): 386 (M+1, 15.37%), 246 (23.45%), 185 (17.73%), 63 (100%).

N-cyclohexyl-5-(1-(6-methoxynaphthalen-2-yl)ethyl)-1,3,4-oxadiazol-2-amine (10a)
A suspension of **(9)** (0.02 mol, 3.8 g) in ethanol (50 ml) was dissolved in aqueous sodium hydroxide (5N) with cooling and stirring resulting in the formation of a clear solution. To this, iodine in potassium iodide solution (5%) was added dropwise with stirring till the color of iodine persisted at room temperature. The reaction mixture was then refluxed for 3-5 hr on water bath. A mixture was then concentrated, cooled, and the solid so obtained was filtered and crystallized from ethanol.

Yield (1.3g, 37%); **m.p**. (145-148 ^{o}C), **Analysis**% for $C_{21}H_{25}N_3O_2$ (351.44) Calcd. (Found.) C: 71.77 (71.51), H: 7.17 (6.97), N: 11.96 (11,63). **IR** (cm^{-1}): 3354 (NH), 2934 (CH-aliphatic), 1567 (C=N). **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.10-1.27 (m, 4H, Cyclohexyl H), 1.45-1.55 (m, 3H, Cyclohexyl H + 3H, CH_3) 1.62-1.87 (m, 4H,

Cyclohexyl H), 3.86 (s, 3H, OCH_3), 4.49 (q, 1H, C<u>H</u>CH_3, J=7.2 Hz), 7.14-7.80, (m, 7H, ArH, NH), **MS** (m/z): 320 (M^+-CH_3O, 15.37%), 212 (33.8%), 185 (100%).

N-cyclohexyl-5-(1-(6-methoxynaphthalen-2-yl)ethyl)-1,3,4-thiadiazol-2-amine (10b)
Compound **(9)** (0.01 mol, 3.8) of was added gradually with stirring to cooled conc. Sulphuric acid (10 ml) during 10 min. The mixture was further stirred for another 5 h in an ice bath. The mixture was then poured over crushed ice with stirring. The solid separated out was filtered, washed with water, dried, and recrystallized from ethanol.

Yield (1.1g, 29.97%); **m.p**. (217-220 ^oC), **Analysis**% for $C_{21}H_{25}N_3OS$ (367.51) Calcd. (Found.) C: 68.63 (68.91), H: 6.86 (6.23), N: 11.43 (11.60). **IR** (cm^{-1}): 3432 (NH), 2938 (CH-aliphatic), 1567 (C=N), 1174 (C=S). **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.04-2.04 (3m, 14H, 11Cyclohexyl H+ 3H, CH_3), 3.85 (s, 3H, OCH_3), 4.49 (q, 1H, C<u>H</u>CH3) 7.64-8.21, (m, 7H, 6ArH+ 1NH), **MS** (m/z): 367 (M^+, 0.29%), 368 (M+1, 0.21%), 368 (M+2,0.09%), 340 (0.89), 212 (19.75), 104 (100%).

4-Substituted-N'-(2-(6-methoxynaphthalen-2-yl)propanoyl)benzenesulfonohydrazide (11a-d) and 4-Substituted-N'-(2-(6-methoxynaphthalen-2-yl)propanoyl) benzo hydrazide (11e,f)
Equimolar amounts of **(8)** (0.01 mol, 2.4 g) and the appropriate benzenesulfonylchloride/ or benzoylchloride (0.01 mol) in pyridine (18 ml) was stirred for half an hour at room temp. The mixture was poured onto ice-cold water and extracted with ethylacetate (3x100 ml). The organic phase was washed with 1N HCl and water, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by recrystallization from ethanol to give our target compounds **11a-f**.

11a: (R=CH_3),*N'-(2-(6-methoxynaphthalen-2-yl)propanoyl)-4-methylbenzene sulfonohydrazide*
Yield (2.1 g, 52.7%); **m.p**. 168-170 ^oC, **Analysis** % for $C_{21}H_{22}N_2O_4S$ (398.48), Calcd. (Found) C: 63.30 (63.40), H: 5.65 (6.10), N: 7.03 (6.89). **IR** (cm^1): 3286 (2 NH), 1688 (CO), **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.24-1.26 (d, 3H, CH_3, J=7.2 Hz), 2.25 (s, 3H, CH_3), 3.61 (q, 1H. CH, J=7.2 Hz), 3.85 (s, 3H, OCH_3), 7.00- 7.02 (d, 2H, $ArH_{b,c}$, J=8.1 Hz), 7.14- 7.55 (m, 6H, ArH), , 7.71- 7.75 (m, 2H, $ArH_{a,d}$), 9.67, 10.25 (2s, 2H, 2NH, D_2O exchangeable). **MS** (m/z): 384 [(M^+ -14 (CH_2), 46.24%), 340 (45.13%), 242 (55.32 %), 184 (100%).

11b: (R=Cl), *4-Chloro-N'-(2-(6-methoxynaphthalen-2-yl)propanoyl)benzene sulfonohydrazide*
Yield (1.9 g, 48%); **m.p**. 177-180^{o}C, **Analysis** % for $C_{20}H_{19}ClN_2O_4S$ (418.89), Calcd. (Found) C: 57.34 (57.51), H: 4.57 (4.73), , N: 6.69 (6.36). **IR** (cm^{-1}): 3333, 3133, (sym & asym NH strech), 1686 (CO), **^{1}HNMR** (DMSO-d_6) δ (ppm): 1.25-1.27 (d, 3H, CH_3, J=7.1 Hz), 3.65-367 (q, 1H. CH, J=7.1), 3.87 (s, 3H, OCH_3), 7.13- 7.27 (m, 3H, ArH), 7.48- 7.52 (m, 4H, ArH) 7.64- 7.74 (m, 3H, ArH), 9.96, 10.35 (2s, 2H, 2NH, D_2O exchangeable). **MS** (m/z): 418 (M^+, 3.70 %), 318 (0..23 %), 244 (1.12 %), 185 (100 %).

11c: (R=Br)**,** *4-Bromo-N'-(2-(6-methoxynaphthalen-2-yl)propanoyl)benzene sulfonohydrazide*
Yield (1.92 g, 48%); **m.p**. 177-180 ^oC, Analysis % for $C_{20}H_{19}BrN_2O_4S$ (462.34), Calcd. (Found) C: 51.84 (51.34), H: 4.13 (4.58), N: 6.05 (6.31). **IR** (cm^{-1}): 3344 (2NH), 1678 (C=O) **^{1}HNMR** (DMSO-d6) δ (ppm) : 1.25-1.27 (d, 3H, CH_3), 3.65-3.67 (q, 1H. C<u>H</u>CH_3), 3.85 (s, 3H, OCH_3), 7.14- 7.56 (m, 6H, ArH), , 7.72- 7.76 (m, 4H, ArH_{a-d}),, 9.96, 10.36 (2s, 2H, 2NH, D_2O exchangeable). **MS** (m/z): 462 (M^+, 2.8 %), 464 (M+2, 2.7 %),due to Br isotope, 141(10.7 %), 185 (100 %).

11d: (R=F), *4-Fluoro-N'-(2-(6-methoxynaphthalen-2-yl)propanoyl)benzene sulfonohydrazide*
Yield (1.2 g, 30%); **m.p**. 117-120 ^oC, **Analysis** % for $C_{20}H_{19}FN_2O_4S$ (402.44), Calcd. (Found) C: 59.69 (59.68), H: 4.76 (4.91), N: 6.96 (6.69). **IR** (cm^{-1}): 3286, (2 NH), 2972(CH-aliphatic) 1688 (CO), **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.42-1.45 (d, 3H, CH_3, J=6.9 Hz), 3.61-3.66 (q, 1H. C<u>H</u>CH_3, J=7.1), 3.85 (s, 3H, OCH_3), 4.21 (s, 1H, NH, D_2O exchangeable), 7.11- 7.14 (d, 2H, ArH_i, J=8.7 Hz), 7.15 (s, 1H, ArH_h), 7.43- 7.46 (d, 2H, ArH_f, J=8.7 Hz), 7.71-7.78 (m, 7H, ArH), 9.19 (s, H, NH, D_2O exchangeable). **MS** (m/z): 402 (M^+, 0.70%), 318 (0..23%), 244 (4.12%), 185 (100%).

11e: (R=H), *N'-(2-(6-methoxynaphthalen-2-yl) propanoyl)benzohydrazide*
Yield (1.51 g, 44.4%); **m.p**. 197-200 ^oC, **Analysis** % for $C_{21}H_{20}N_2O_3$ (348.40), Calcd. (Found) C: 72.40 (72.78), H: 5.79 (5.11), N: 8.04 (8.21). **IR** (cm^{-1}): 3305, 3197 (sym & asym NH strech), 1682, 1653 (2CO), **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.23-1.26 (d, 3H, CH_3, J=7.2 Hz), 3.65-3.68 (q, 1H. C<u>H</u>CH_3, J=9.6), 3.86 (s, 3H, OCH_3), 7.13-7.75

(m, 10H, ArH), 9.82, 10.29 (2s, 2H, 2NH, D_2O exchangeable). **MS** (m/z): 348 (M^+, 8.46%), 349 (M^{+1}, 1.08%), 350 (M^{+2}, 0.25%), 185 (100%).

11f: (R=Cl), *4-Chloro-N'-(2-(6-methoxynaphthalen-2-yl)propanoyl)benzohydrazide*, **Yield** (1.71 g, 45%); **m.p**. 167-170 ^oC, **Analysis** % for $C_{21}H_{19}ClN_2O_3$ (382.84), Calcd. (Found) C: 65.88 (65.67), H: 5.00 (4.98), N: 7.32 (7.09). **IR** (cm^{-1}): 3200 (2NH), 1672 (C=O). **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.37-1.39 (d, 3H, CH_3), 3.86 (s, 3H, OCH_3), 3.88-3.92 (q, 1H, C<u>H</u>CH_3), 7.13-7.87 (m, 10H, ArH), 10.18, 10.42 (2s, 2H, 2NH, D_2O exchangeable). **MS** (m/z): 383 (M^+, 5.25%), 302 (4.16%), 139 (81.70%), 110 (100%).

Ethyl 4-(2-(6-methoxynaphthalen-2-yl)propanamido)benzoate (12)
A mixture of equimolar amounts (0.01 mol, 2.4 g) of 2-(6-methoxy-2-napthyl)propanoic acid chloride (**2)** and ethyl 4-aminobenzoate (0.01 mol, 1.6 g) in DMF (10 mL) containing few drops of pyridine was refluxed for 2 h. The reaction mixture was poured over crushed ice, few drops of HCl was added and the separated solid product was filtered, dried and recrystallized from ethanol.

Yield (1.42g, 38%); **m.p**. (152-155 ^oC), **Analysis**% for $C_{23}H_{23}NO_4$ (377.43) Calcd. (Found.) C: 73.19 (73.39), H: 6.14 (6.45), N: 3.71 (3.58). **IR** (cm^{-1}): 3110 (NH), 1712 (COO), 1656 (CONH), **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.25-1.31 (d, 3H, CH_3 ester, J=7.3 Hz), 1.48-1.52 (m, 3H, CHC<u>H</u>$_3$, J=7.1 Hz), 3.88 (s, 3H, OCH_3), 3.97-4.01 (m, 1H. C<u>H</u>CH3, J=7.1), 4.23-4.30 (m, 2H. CH_2 ester, J=7.3), 3.87 (s, 3H, OCH_3), 7.12- 7.15, (d, 1H, Ar_i), 7.27(s, 1H, ArH_h) 7.48- 7.52, (d, 1H, ArH_f), 7.71- 7.93 (m, 7H, ArH), 10.40 (s, 1H, NH, D_2O exchangeable). **MS** (m/z): 377 (M^+, 1.24%), 302 (3.14%), 185 (100%).

N-(4-(hydrazinecarbonyl)phenyl)-2-(6-methoxynaphthalen-2-yl)propanamide (13)
A mixture of (0.01 mol, 3.7 g) of **12** and hydrazine hydrate (0.05 mol) in absolute ethanol (20ml) was refluxed for 10 h. The mixture was concentrated, cooled and poured in crushed ice in small portions while stirring and kept for 3-4 h at room temperature. The solid so obtained was filtered, dried and crystallized from ethanol.

Yield (2.44g, 66%); **m.p**. (200-203 ^oC), **Analysis**% for $C_{21}H_{21}N_3O_3$ (363.41) Calcd. (Found.) C: 69.41 (68.94), H: 5.82 (5.42), N: 11.56 (11.28). **IR** (cm^{-1}): 3296 (NH), 1652 (CONH), **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.47-1.49 (d, 3H, CH_3, J=6.9 Hz), 3.90 (s, 3H, OCH_3) 4.02-4.07 (q, 1H. C<u>H</u>CH_3, J=7.1), 7.11- 7.15, (d, 2H, ArHa,d), 7.26- 7.31, (m, 3H, ArH), 7.47- 7.52, (d, 2H, ArHb,c), 7.69- 7.80, (m, 3H, ArH), 8.82 (brs, 2H, NH_2 D_2Oexchangeable), 10.53 (s, 1H, NH D_2O exchangeable). **MS** (m/z): 363 (M^+, 3.14%), 349 (2.23%), 333 (11.85 %), 185 (100%).

N-(4-(2-(cyclohexylcarbamothioyl) hydrazinecarbonyl) phenyl)-2-(6-methoxynaphthalen-2-yl)propanamide (14)
A mixture of equimolar amounts of compound (**13**) (0.01 mol, 3.6 g) and cyclohexyl isothiocyanate (0.01 mol, 1.4 g) in dioxane (20 ml) was refluxed for 8 h on water bath. The reaction mixture was then concentrated, cooled, and kept overnight in the refrigerator. The solid separated out was filtered, dried, and crystallized from ethanol.

Yield (1.76g, 35%); **m.p**. (117-120 ^oC), **Analysis**% for $C_{28}H_{32}N_4O_3S$ (504.64) Calcd. (Found.) C: 66.64 (66.88), H: 6.39 (6.56), N: 11.10 (11.40). **IR** (cm^{-1}): 3252 (3NH), 2932 (CH-aliphatic), 1664 (CO), 1178 (C=S). **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.10-1.24 (m, 6H, Cyclohexyl H), 1.49-1.51 (m, 3H, CH_3), 1.76-1.85 (m, 5H, Cyclohexyl H), 3.85 (s, 3H, OCH_3), 3.97-3.99 (q, 1H, C<u>H</u>CH_3), 6.70 (s, 1H, NH), 7.12-7.85, (m, 10H, ArH) 9.09 (s, 1H, CSNH D_2O exchangeable), 10.10, 10.32 (2s, 2H, 2CONH, D_2O exchangeable), **MS** (m/z): 504 (M^+, 1.38%), 426(1.38%), 185(57.97%), 115 (100%).

N-(4-(5-(cyclohexylamino)-1,3,4-oxadiazol-2-yl)phenyl)-2-(6-methoxynaphthalen-2-yl)propanamide (15)
A suspension of compound **(14)** (0.002 mol, 1.2 g) in ethanol (50 ml) was dissolved in aqueous sodium hydroxide (5N) with cooling and stirring resulting in the formation of a clear solution. To this, iodine in potassium iodide solution (5%) was added dropwise with stirring till the color of iodine persisted at room temperature. The reaction mixture was then refluxed for 5 h on water bath. A mixture was then concentrated, cooled, and the solid so obtained was filtered, dried and recrystallized from ethanol.

Yield (1 g, 24.69%); **m.p**. (157-160 ^oC), **Analysis**% for $C_{28}H_{30}N_4O_2S$ (486.63) Calcd. (Found.) C: 69.11 (68.79), H: 6.21 (5.81), N: 11.51 (11.61), **IR** (cm^{-1}): 3290 (NH), 2930 (CH-aliphatic), 1658 (CON), 1520 (C=N). **^{1}HNMR** (DMSO-d_6) δ (ppm) : 0.89-2.08 (3m, 14H, 11Cyclohexyl H + 3H CH_3), 3.83 (s, 3H, OCH_3), 4.28 (q, 1H,

C<u>H</u>CH_3), 7.12-7.77 (m, 10H, ArH) 10.37 (s, 2H, SH+NH D_2O exchangeable), **MS** (m/z): 486 (M^+, 14.7%), 474 (17.6%), 185 (6.38%), 66 (100%).

N-(4-(4-cyclohexyl-5-mercapto-4H-1,2,4-triazol-3-yl)phenyl)-2-(6-methoxynaphthalen-2-yl)propanamide (16)
A suspension of of compound **(14)** (0.002 mol, 1.2g) in ethanol (50 ml) was dissolved in aqueous sodium hydroxide (4N), resulting in the formation of a clear solution.The reaction mixture was refluxed for 6 h on a water bath, concentrated, cooled, and filtered. The pH of the filtrate was adjusted with acetic acid to 5-6 and kept aside for 1-2 hr. The solid separated out was filtered, washed with water, dried, and crystallized from ethanol.

Yield (1 g, 24.69%); **m.p**. (157-160 ^oC), **Analysis**% for $C_{28}H_{30}N_4O_2S$ (486.63) Calcd. (Found.) C: 69.11 (68.79), H: 6.21 (5.81), N: 11.51 (11.61). **IR** (cm^{-1}): 3290 (NH), 2930 (CH-aliphatic), 1658 (CON)., 1520 (C=N). **^{1}HNMR** (DMSO-d_6) δ (ppm) : 0.89-2.08 (3m, 14H, 11Cyclohexyl H + 3H CH_3), 3.83 (s, 3H, OCH_3), 4.28 (q, 1H, C<u>H</u>CH_3), 7.12-7.77 (m, 10H, ArH) 10.37 (s, 2H, SH+NH D_2O exchangeable), **MS** (m/z): 486 (M^+, 14.7%), 474 (17.6%), 185 (6.38%), 66 (100%).

N,N'-(Subsituted-phenylene)bis(2-(6-methoxynaphthalen-2-yl)propanamide)(17a,b)
Equimolar amounts of naproxen acid chloride **(2)** (0.01 mol, 2.4 g)) and o or p-phenylene diamine (0.01 mol, 1.08 g) in DMF (20 ml) containing few drops of pyridine was stirred at room temperature for 2 h. The reaction mixture was then poured on to ice-cold water and HCl, the solid product so formed was filtered, dried and recrystallized from ethanol.

17a: *N,N'-(1,2-phenylene)bis(2-(6-methoxynaphthalen-2-yl)propanamide*
Yield (0.91g, 40%); **m.p**. (137-140^{o}C), **Analysis** % for $C_{34}H_{32}N_2O_4$ (532.63), Calcd. (Found) C: 76.67 (76.60), H: 6.06 (5.90), N: 5.26 (5.58). **IR** (cm^{-1}): 3300 (NH), 2978 (CH-aliphatic) 1656 (C=O), **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.43-1.45 (d, 3H, CH_3, J=6.9 Hz), 3.76-3.78 (q, 1H, C<u>H</u>CH_3, J=6.9), 3.83 (s, 3H, OCH_3), 7.13-7.16 (m, 3H, ArH), 7.27-7.41 (m, 7H, ArH), 7.70-7.80 (m, 6H, ArH), 10.24 (s, 2H, 2NH D_2O exchangeable). **MS** (m/z): 532 (M^+, 19.0%), 297 (19.0 %), 185 (66.7%), 77(100%).

17b:,*N,N'-(1,4-phenylene)bis(2-(6-methoxynaphthalen-2-yl)propanamide)*
Yield (0.88 g, 39%), **m.p**. (207-210^{o}C), **Analysis** % for $C_{34}H_{32}N_2O_4$ (532.63), Calcd. (Found) C: 76.67 (76.51), H: 6.06 (6.23), N: 5.26 (5.51). **IR** (cm^{-1}): 3392 (NH), 1660 (C=O), **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.43-1.46 (d, 3H, CH_3, J=6.9 Hz), 3.83 (s, 3H, OCH_3), 3.91-3.93 (q, 1H. C<u>H</u>CH_3, J=6.9), 7.11-7.80 (m, 16H, ArH), 9.99 (s, 2H, 2NH D_2O exchangeable). **MS** (m/z): 532 (M^+, 5.06%), 533 (M+1, 4.13%), 398 (0.85 %), 320 (7.71%), 185(100%).
4-(2-(6-methoxynaphthalen-2-yl)propanamido)-N-(4-(N-substituted sulfamoyl)phenyl)benzamide (18a,b)

Equivalent amounts of compound (**12)** (0.01 mol, 1.85 g) and sulphanilamide (0.01 mol, 1.7g) or sulphadiazine (0.01 mol, 2.5 g) in few drops of pyridine was fused together. The formed mass was then triturated with ethanol and the separated crystals were recrystalized by boiling several times with ethanol to give **18a** and **18b** respectively.

18a: (R=H), *4-(2-(6-methoxynaphthalen-2-yl)propanamido)-N-(4-sulfamoyl lphenyl)benzamide*
Yield (0.9 g, 33%); **m.p**. (205-207 ^oC), **Analysis** % for $C_{27}H_{25}N_3O_5S$ (503.57) Calcd. (Found.) C: 64.40 (64.65), H: 5.00 (5.20), N: 8.34 (8.54). **IR** (cm^{-1}): 3286, 3400 (NH, NH_2), 1656 (CONH), **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.48-1.50 (d, 3H, CH_3, J=7.2 Hz), 3.85 (s, 3H, OCH_3) 4.10-4.12 (q, 1H. CH, J=7.1), 7.12- 7.82, (m, 14H, ArH), 9.86 (d, 2H, NH_2, D_2O exchangeable), 10.32, 10.43 (2s, 2H, 2NH, D_2O exchangeable). **MS** (m/z): 348 (M^+-$C_6H_7NSO_2$, 1.24%), 384 (15.09%), 185 (100%).

18b: (R =2-Pyrimidine), *4-(2-(6-methoxynaphthalen-2-yl)propanamido)-N-(4-(N-pyrimidin-2-ylsulfamoyl) phenyl) benzamide*
Yield (1.08 g, 34.44 %); **m.p**. (177-180 ^oC), **Analysis** % for $C_{31}H_{27}N_5O_5S$ (581.64) Calcd. (Found.) C: 64.01 (64.43), H: 4.68 (5.15), N: 12.04 (12.00). **IR** (cm^{-1}): 3202 (NH), 1656 (CONH), **^{1}HNMR** (DMSO-d_6) δ (ppm) : 1.47-1.51 (d, 3H, CH_3, J=7.1 Hz), 3.85 (s, 3H, OCH_3), 4.25-4.27 (q, 1H, CH, J=7.1 Hz), 7.12- 7.82, (m, 16H, ArH), 9.00, 10.09, 10.43 (3s, 3H, 3NH, D_2O exchangeable). **MS** (m/z): 581 (M^+, 0.22%), 474 (0.24%), 185 (74%) 119 (100%).

Anti-tumor screening test.

Activity against human cancer colon cell line HCT-116

Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentrations of the compounds under test (0-36.00 μM/mL) were solubilised in dimethyl-sulfoxide (DMSO) and were added to the cell monolayer of the five human cell lines. Monolayer cells were incubated with the compounds for 48 h at 37°C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with sulforhodamine B stain. The color intensity was measured in an ELISA reader [14]. The relation between surviving fraction and drug concentration is plotted to get the survival curve for the active compounds. Statistical Analysis Student's t test was used for analysis of the biochemical parameters. The data were expressed as mean ±standard error.

Docking using MOLSOFT ICM 3.4-8C program [15]

1- Convert our PDB file into an ICM object: This conversion involves addition of hydrogen bonds, assignment of atoms types, and charges from the residue templates.

2- To perform ICM small molecule docking:

a) Setup docking project:

1) Set project name:

2) Setup the receptor:

3) Review and adjust binding site:

4) Make receptor maps:

b) Start docking simulation:

3- Display the result:

ICM stochastic global optimization algorithm attempts to find the global minimum of the energy function that include five grid potentials describing interaction of the flexible ligand with the receptor and internal conformational energy of the ligand, during this process a stack of alternative low energy conformations is saved (Table 3).The mode of interaction of the (indomethacin) within (1S2A) was used as a standard docking model. All inhibitors were compared according to the best binding free energy (minimum) obtained among all the run.

RESULTS AND DISCUSSION

Chemistry

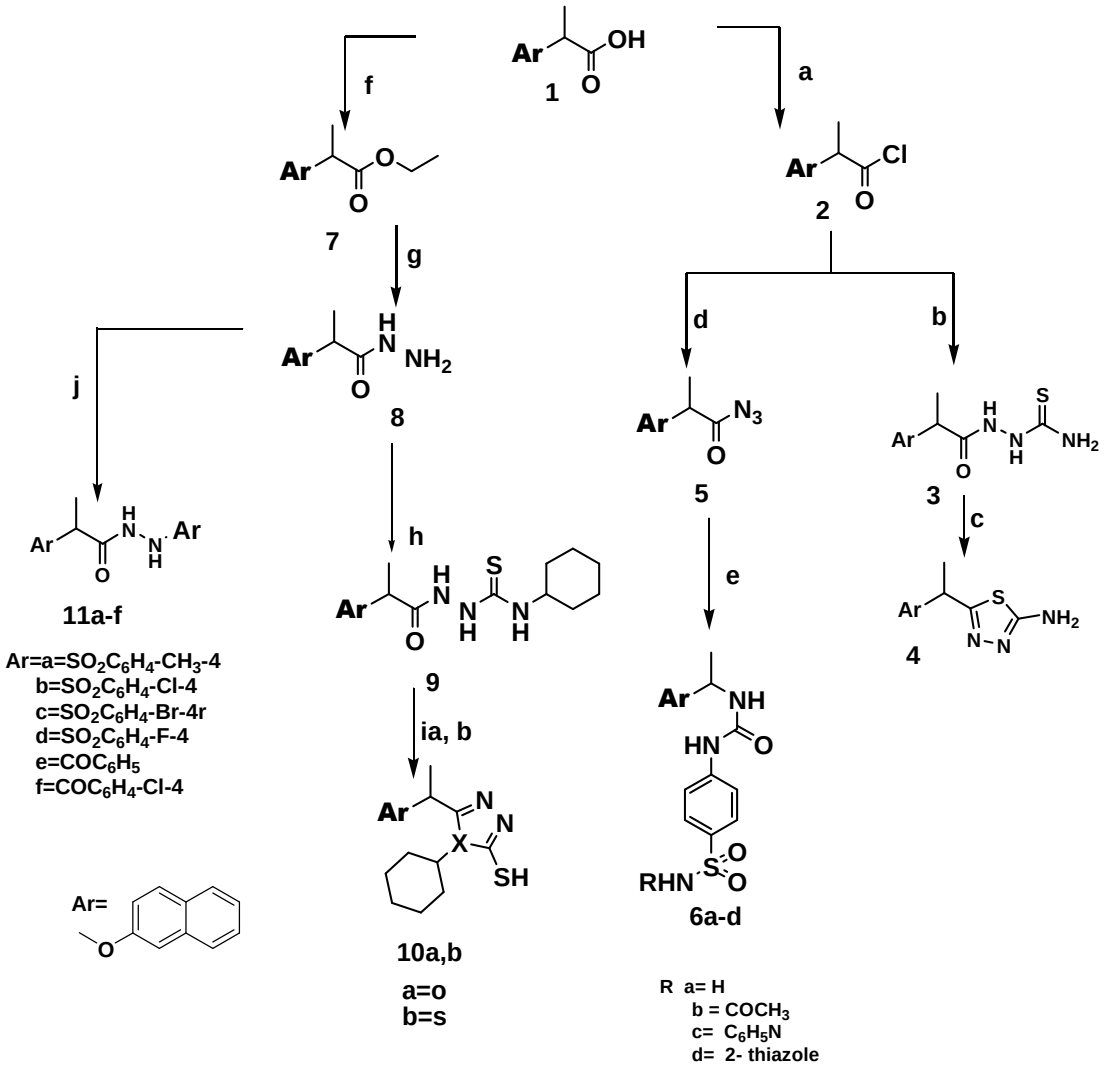
Naproxen acid chloride **2** is considered as a key starting material in the present study. It was converted to the target compound **3** through reaction with nucleophilic reagent as thiosemicarbazide in the presence of pyridine as a base. Cyclocondensation was achieved through heating under reflux with phosphorous oxychloride to give 5-(1-(6-Methoxynaphthalen-2-yl)ethyl)-1,3,4-thiadiazol-2-amine .

Reaction of **2** with sodium azide in DMF produced the azido derivative **5**. when the azido derivative was refluxed with different sulphonamides, the urea derivatives were obtained through the curtius rearrangement [10], where the amino of sulfonamides react with the intermediate (isocyanates) to form the urea derivatives **6a-d** . Esterification of naproxen with ethanol afforded the ester derivative **7**, hydrazinolysis of the latter using ethanolic hydrazine hydrate gave the hydrazide **8**. Reaction of **8** with cyclohexyl isothiocyanate yeilded the corresponding thiosemicarbazide derivative **9** [16]. Oxidative cyclisation of compound **9** to 1,3,4 oxadiazole derivatives **10a** by elimination of H_2S using iodine and potassium iodide in ethanolic sodium hydroxide. However, N-cyclohexyl-5-(1-(6-methoxynaphthalen-2-yl)ethyl)-1,3,4-thiadiazol-2-amine **(10b)** was obtained by cyclization of **9** by treating with cold concentrated sulphuric acid [17-20]. The addition reaction of the hydrazide **8** with the different sulphonyl/ benzoyl chlorides in the presence of pyridine as a base afforded a series of 1-pyrazolonyl-2(1H)-pyridones **11a-f**. The reaction proceeded initially by the nucleophilic attack of substituted/unsubstituted benzene sulphonyl chloride or benzoyl chloride by the β hydrazine nitrogen, followed by the formation of an intermediary hydrochloride complex. This complex is more or less rapidly dehydrochlorinated by the presence of base which used as HCl acceptor [21].

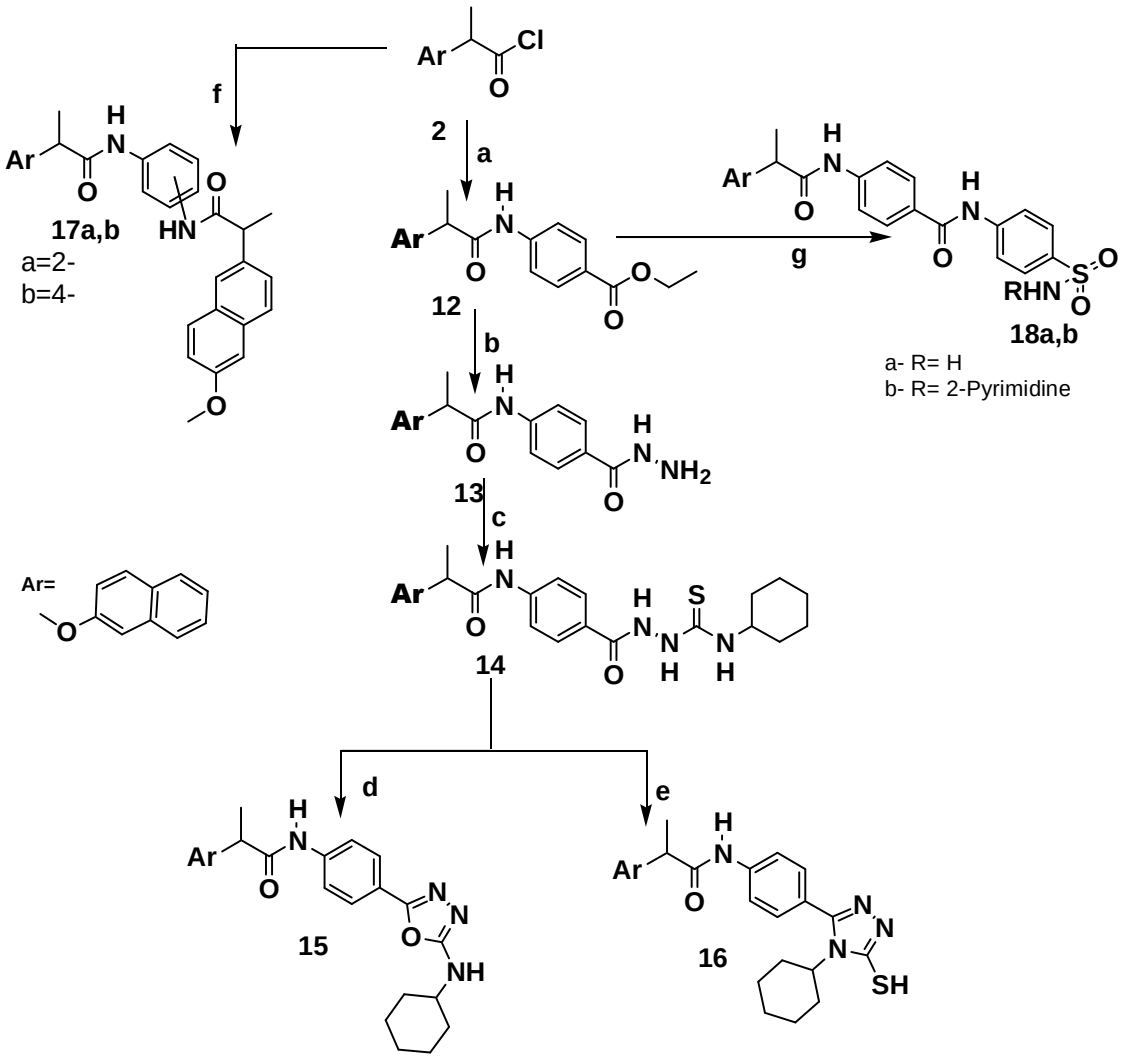
Accordingly, acid chloride **2** was allowed to react with 4-aminoethylbenzoate via nucleophilic addition. Hydrazinolysis of the produced ester **12** using ethanolic hydrazine hydrate afforded the hydrazide derivative **13,** which was allowed to react with cyclohexyl isothiocyanate to yeild the corresponding thiosemicarbazide derivative **14.**

1,3,4-oxadiazol-2-amine **15** and 1,2,4-triazole-3-thione **16** were obtained by oxidative cyclisation to **15** by elimination of H_2S using iodine and potassium iodide in ethanolic sodium hydroxide or smooth cyclization through dehydration with 4N NaOH in ethanol to give **16** respectively.

The nucleophilic addition of amino group of 1 and 3-phenylenediamines to the carbonyl group of acid chloride **2** afforded the bis(2-(6-methoxynaphthalen-2-yl)propanamide)derivatives **17a,b**. Fusion of the ester compound **12** with two different sulfonamides in the presence of two drops of pyridine afforded compounds **18a,b**.



Scheme 1, Reagents and conditions:
a; */SOCl*$_2$*/benzene, reflux,* ***b***; *NH*$_2$*NHCSNH*$_2$*/DMF, reflux,* ***c***: *POCl*$_3$*/ reflux,*
d;*NaN*$_3$*/Acetone,stirring,****e***;*NH*$_2$*C*$_6$*H*$_4$*SO*$_2$*NHR/pyridine,reflux,****f***;*EtOH/H*$_2$*SO4conc.,reflux,****g***;*NH*$_2$*NH*$_2$*/EtOH,reflux,* ***h***;*C*$_6$*H*$_{12}$*N=C=S/dioxane,* ***i:a***;*5 N.NaOH,EtOH/KI stirring,* ***i:b***; *H*$_2$*SO*$_4$ *conc. stirring .****j;*** *ArSO*$_2$*Cl or ArCOCl/Pyridine, stirring.*



Scheme 2, Reagents and conditions:

a:$NH_2C_6H_4CO_2Et$, *reflux* ***b***:NH_2NH_2/ *EtOH, Reflux,* ***c***:$C_6H_{11}NCS$/*Dioxane,reflux,* ***d***:*5N NaOH/* I_2*in KI/ EtOH,* ***e;****4N NaOH/EtOH,* ***f****; o/ p,*$NH_2C_6H_4NH_2$*/DMF, stirring,* ***g****;* $NH_2C_6H_4SO_2NHR$*/pyridine, fusion.*

Biological evaluation:

Antitumor screening test:

In order to appreciate the actual utility of our newly synthesized compounds in cancer chemotherapy, in vitro cytotoxicity were carried out. Development therapeutic program (DTP), Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI), Betheda, Maryland, USA has adopted an *in-vitro* model consisting of 60 human cell lines for primary anticancer screening. Screening utilizes 60 different human tumor cell lines, representing leukemia, melanoma and cancers of lung, colon, brain, ovary, breast, prostate and kidney. It is a unique screen in that the complexity of 60 cell line dose response produced by a given compound results in a biological response pattern which can be utilized in pattern recognition algorithms (COMPARE program). Using these algorithms, it is possible to assign a putative mechanism of action to a test compound, or to determine that the response pattern is unique and not similar to that of any of the standard prototype compounds included in the NCI database.

Eleven compounds **4, 10a,b, 6a-d, 15, 16, and 18a,b** were selected by NCI to be evaluated in the 60 cell line panel

The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10 µM. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE program. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels (0.01–100 µM).

Table 1. Growth inhibitory activity (GI_{50}) of some selected in vitro tumor cell lines (µM)[a]

Cpd No	NCS	Panel	Subpanel cell line	Growth %
6b	751311	Non small cell lung Cancer	NCI-H522	44.24
		Colon Cancer	HCT-116	37.95
6c	571308	Non small cell lung Cancer	NCI-H522	63.09
		Colon Cancer	HCT-116	35.53
		Renal cancer	RXF393	42.55
6d	751309	Non small cell lung Cancer	A549/ATCC	61.57
			NCI-H522	51.73
		Colon Cancer	HCT-116	35.40
		CNS Cancer	SNB-75	50.70
			U251	66.34
		Ovarian Cancer	OVCAR-8	65.32
16	751884	Leukemia	CCRF-CEM	64.41
			K-562	63.89
			MOLT-4	55.94
			RPMI-822	54.26
		Non small cell lung Cancer	A549/ATCC	41.22
			HOP-92	43.42
			NCI-H460	43.24
			NCI-H522	64.77
		Colon Cancer	HCT-116	48.97
		CNS Cancer	SF-268	65.50
			SF-295	60.69
			SNB-75	67.00
			U251	56.83
		Melanoma	M14	63.17
			MDA-MB-435	62.43
			SK-MEL-5	67.51
			UACC-62	58.29
		Ovarian Cancer	NCI/ADR-RES	50.74
		Renal Cancer	CAKI-1	44.73
		Prostate Cancer	PC-3	39.18
		Breast Cancer	MCF7	48.27

[a]*Data obtained from NCI in vitro disease-oriented human cell screen*
**means that growth percent was more than 70%*

IC50 activity against Colon cancer cell line HCT-116

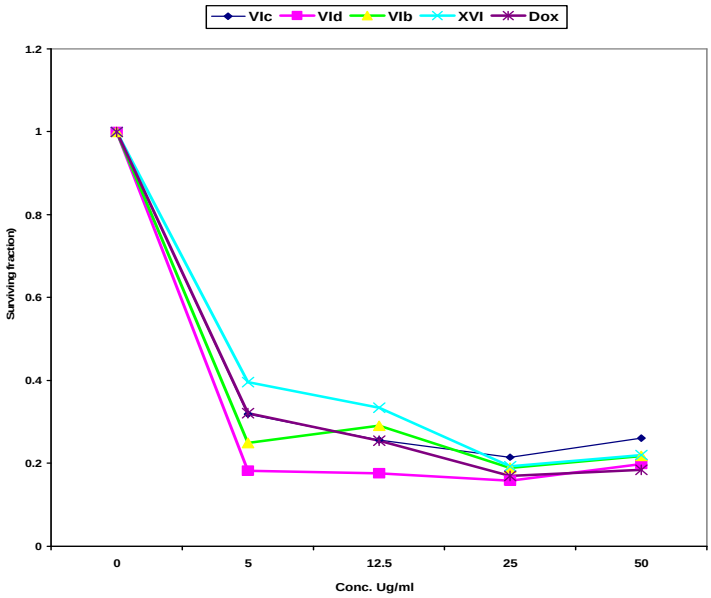
Unfortunately none of our tested compounds were selected for five dose tests, but Compounds **6b, c, d, 16** were found to have promising inhibitory activity against colon cancer cell line HCT-116, growth percentage in the range of 35.4-48.9 %. The cytotoxic effects of the four compounds and doxorubicin, as a reference drug, in five different concentrations, were evaluated in the National Institute of Cancer, Cairo, Egypt.

Table 2. In vitro cytotoxic activity of selected synthesized compounds of colon cancer cell line(HCT 116).

Cpd No	Surviving fraction concentration					IC_{50} µM
	0	5	12.5	25	50	
6b	1.00	0.32	0.56	0.22	0.26	8.09
6c	1.00	0.18	0.18	0.16	0.20	6.57
6d	1.00	0.25	0.29	0.19	0.22	7.10
16	1.00	0.40	0.33	0.19	0.22	8.30
Ref*	1.00	0.32	0.26	0.17	0.18	7.10

Ref=Doxorubicin*
IC_{50}= compound concentration required to inhibit tumor cell line proliferation by 50 %.

Fig. 2.Cytotoxic activity of the tested compounds and doxorubicin on cell survival of Colon Cancer cell line (HCT-116)



Potential cytotoxicity of the active compounds was tested using the method of Skehan et al [14,22]. Colon cancer cell line HCT-116 were incubated with five concentrations (0-50) µM/ml for each compound. All the tested compounds showed selective cytotoxicity against the colon cancer cell line (HCT-116) superior or compared to doxorubicin, with IC50 values of 8.09, 6.57, 7.10, 8.30, 7.10 (µM) respectively. The growth inhibitory action of the selected compounds is summarized in Table -2 and Fig-2

Molecular Modeling Studies.

To pre-asses the anti-tumorigenic behavior of our 6-methoxynapthalene derivatives **6b-d**, **16** on a structural basis, automated docking studies were carried out using MOLSOFT ICM 3.4-8C program [19] the scoring functions and hydrogen bonds formed with the surrounding amino acids are used to predict their binding modes, their binding affinities and orientation of these compounds at the active site of AKR1C3 enzyme. The protein–ligand complex was constructed based on the X-ray structure (PDB entry 1S2A) AKR1C3 with its bound inhibitor indomethacin [5]. The scoring functions of the compounds were calculated from minimized ligand protein complexes. The X-ray crystal structure of AKR1C3 reveals a substrate-binding site that consists mainly of: hydrophobic aromatic amino acid side chains (Tyr24, Tyr55, Leu54,Trp227, and Phe306). An oxyanion hole, which is located at the bottom of the hydrophobic pocket, is formed by active site tyrosine (Tyr55), histidine (His117), and the coenzymes nicotinamide ring [9].

In our investigation, the 3D-coordinates in X-ray crystal structure of AKR1C3 in complex with the ligand, indomethacin (PDB entry 1S2A) [5] was used as the receptor model in AKR1C3 docking simulation. The docked model of indomethacin with AKR1C3 (Fig-3) was consistent with the previously reported X –ray analysis [5] and revealed the following binding mode: The carbonyl oxygen is far away from Tyr 55 or His 117 to H-bond directly. Instead, the carboxylate group points toward and interacts with the oxygen atoms O1n, O2n from the nicotinamide half of the NADP+ diphosphate moiety, forming two hydrogen bonds, and additional H-bond if formed between indomethacin O2 of COOH and He2 of Glu 222.

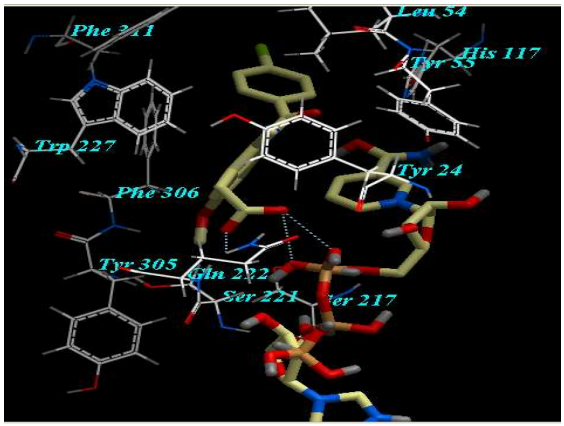


Fig. 3. The proposed binding mode of original ligand indomethacin into the active binding site of AKR1C3 active site. It has ICM score of -80.45, it forms three hydrogen bonds, two of them between H of COOH and O1n,O2n of Phosphate moiety of NADP. And another hydrogen bond between O2 of COOH and He2 of amino acid Glu222…

Also our docking studies revealed that, naproxen occupies a similar position of active site as indomethacin (fig-4). It's O2 of carboxylate could form H-bonds with the H1n1 atom of NADP+ diphosphate moiety, in addition to two hydrogen bonds between O3 of carboxylate and Hn, Hb1 of amino acids Tyr23, Tyr24 respectively.

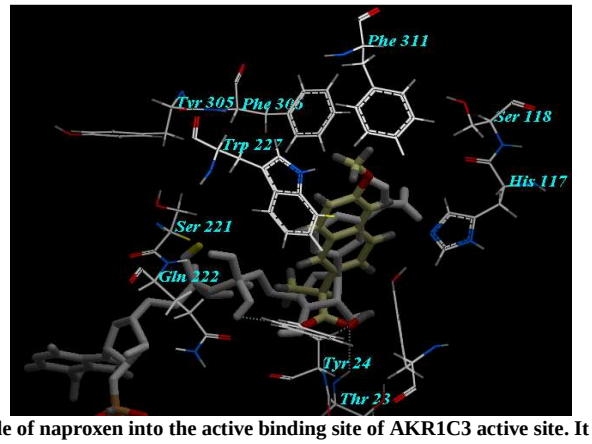


Fig. 4. The proposed binding mode of naproxen into the active binding site of AKR1C3 active site. It has ICM score of -73.44, it forms three hydrogen bonds, one of them between O2 of COOH and H1n1 of Phosphate moiety of NADP. Another two hydrogen bond between O3 of COOH and Hn, Hb1, of amino acids Tyr23, Tyr24 respectively…

Our active compounds **6b, c, d and 16** when modeled in the active site of AKR1C3 enzyme. (Table 3, figs 5-8) revealed strong binding affinities, their binding energies were -132.88, -137.22, -137.69, -102.60 Kcal/mol, respectively compared to -80.45 &-73.44 of indomethacin & naproxen respectively. It is interesting to point out that compounds **6b, c, d and 16** were found to be a very promising AKR1C3 inhibitors, as it takes advantage by it's ability to H bond to amino acid present in the oxyanion hole (Tyr 55 and His 117) in the vicinity of the coenzyme's nicotinamide with the aromatic residues located in the hydrophobic pocket, and at the same time to NADP+ diphosphate.

Table. 3. ICM scores of naproxen, tested compounds, indomethacin, and hydrogen bonds formed with amino acid residues and their lengths.

Cpd No	Docking Score Kcal/mol	No of Hydrogen bonds	Atom of ligand involved	Amino acid residues forming the hydrogen bonds	Length of hydrogen bond (A)
Naproxen	**-73.44**	3	O2 of COOH O3 of COOH O3 of COOH	Phosphate moiety of NADP….H1n1 Tyr24…Hb1 Tyr23…..Hn	2.22 1.82 2.18
6b	**-132.88**	5	O3 of SO_2 O4 of SO_2 O5 of $COCH_3$ H11 of CONH H23 of $CHCH_3$	Phosphate moiety of NADP….H1n1 Phosphate moiety of NADP….H2a1 Glu222… Hg1 Tyr 55…Oh Amide of Nicotin-amide ring.…O7n	2.57 2.46 1.94 1.51 2.52
6c	**-137.22**	4	O2 of CONH O2 of CONH O3 of SO_2 O4 of SO_2	Tyr 55…He2 His117…..He1 Glu222… Hg1 Phosphate moiety of NADP….H1n1	2.56 2.69 2.01 2.27
6d	**-137.69**	5	O1 of OCH_3 O4 of SO_2 O4 of SO_2 H15 of $C_6H_5SO_2$ H14 of $C_6H_5SO_2$	Phosphate moiety of NADP….Ha1 His117…..Hd2 Amide of Nicotin-amide ring.…H7n7 Tyr 55…He2 Tyr 55…He2	2.80 2.65 2.77 2.66 1.94
16	**-102.60**	5	O1 of CONH O1 of CONH O2 of OCH_3 N2 of Triazole N3 of Triazole	Tyr 55…Hd1 Amide of Nicotin-amide ring.…H7n2 Phosphate moiety of NADP….Ha1 Glu222… Hn Glu222… Hn	2.19 2.07 2.37 2.50 2.80
Indomethacin	-80.45	3	H of COOH H of COOH O2 of COOH	Phosphate moiety of NADP….O1n Phosphate moiety of NADP….O2n Glu222… He2	2.36 1.57 2.24

Fig 5-8. Binding modes of compounds 6 b, c, d and 16 into the binding site of AKR1C3.

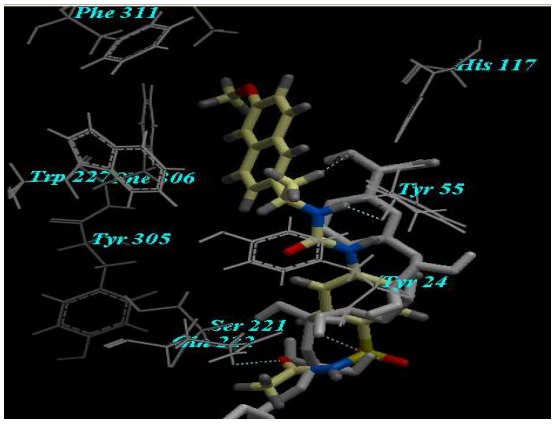


Fig. 5. The proposed binding mode of 6b into the active binding site of AKR1C3 active site. It has ICM score of -132.88, it forms five hydrogen bonds, two of them between O3, O4 of SO2 and H1n1, H2a1 of Phosphate moiety of NADP. One hydrogen bond between O5 of $COCH_3$ and Hg1 of amino acid Glu 222, other hydrogen bond between H11 of CONH and Oh of Tyr55. The last hydrogen bond is between H23 of CHCH3 and O7n of Amide of Nicotinamide ring….

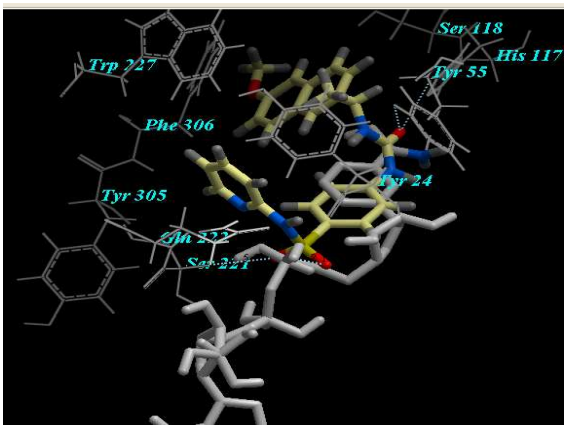


Fig. 6. The proposed binding mode of 6c into the active binding site of AKR1C3 active site. It has ICM score of -137.72, it forms four hydrogen bonds, two of them between O2 of CONH and He2 and He1 of Tyr55 and His117 respectively…One hydrogen bond between O3 of SO_2 and Hg1 of amino acid Glu 222. And hydrogen bond between O4 of SO2 and H1n1 of Phosphate moiety of NADP….

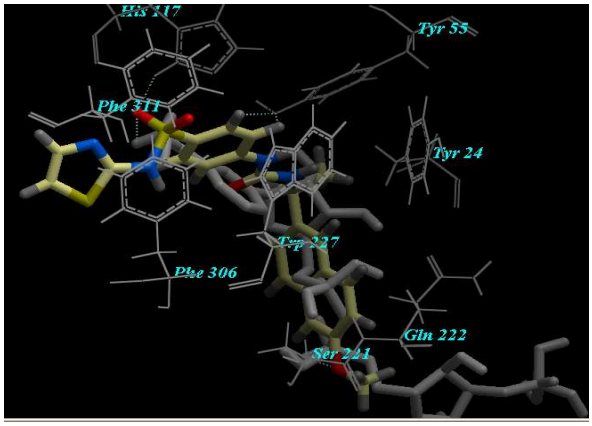


Fig. 7. The proposed binding mode of 6d into the active binding site of AKR1C3 active site. It has ICM score of -137.69, it forms five hydrogen bonds, one between O1 of OCH_3 and Ha1 of Phosphate moiety of NADP. Two hydrogen bonds between O4 of SO_2 and Hd2 of His117 and H7n7 of Amide of Nicotinamide ring …Another two hydrogen bonds is between H14, H15 of benzene ring of sulfonamide and He2 of amino acid Tyr55….

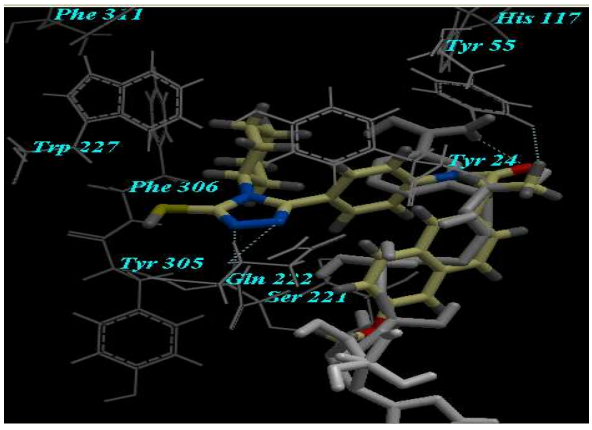


Fig. 8. The proposed binding mode of 16 into the active binding site of AKR1C3 active site. It has ICM score of -102.60, it forms five hydrogen bonds, two between O1 of CONH and Hd1 of Tyr55 and H7n2 o of Amide of Nicotinamide ring. Another one between O2 of OCH_3 and Ha1 of Phosphate moiety of NADP. Two hydrogen bonds between N2, N3 of Triazole and Hn of amino acid Glu 222.

CONCLUSION

In this present study, some functionally derived 6-methoxynapthalenes **2-18** were synthesized, eleven compounds were selected and screened in NCI (National cancer institute, Maryland, USA) to be evaluated in the 60 cell line panel. Unfortunately, none of the compounds was selected to the five dose assay, however some of the compounds **6b-d and 16** showed good growth percentage inhibition especially against colon cancer (HCT-116) ranging from 35.4-48.9 %, these compounds were sent to National cancer institute, Cairo, Egypt, where the in vitro cytotoxicity against HCT 116 cell line of colon cancer was evaluated. IC50 of selected compounds were superior / comparable to standard doxorubicin, values were of 8.09, 6.57, 7.10, 8.30, 7.10 (µM) respectively. Using Molsoft ICM 3.4-8C program, molecular modelling and docking studies of the synthesized compounds into ARK1C3 complexed with its bound inhibitor indomethacin (1S2A) were performed in order to predict the binding affinities and orientations of these compounds at the active site. The ICM score values of **6b-d and 16** were 132.88, -137.22, -137.69, -102.60 Kcal/mol, compared to -80.45 &-73.44 of indomethacin and naproxen respectively.

These ICM values and number of hydrogen bonding showed good agreement with predicted binding affinities as verified by biological data. According to these results, we can conclude that **6b-d and 16** appears to be potentially attractive chemotherapeutic agents especially in colon cancer therapy.

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