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Design and Synthesis of Pyridine-amide Based Compounds Appended Naproxen Moiety as Anti-Microbial and Anti-Inflammatory Agents

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ABSTRACT

A series of pyridinamide-containing naproxen derivatives were synthesized through different routes starting with naproxenoyl chloride (2) which, upon reaction with 2-or 3-aminopyridine, gave the corresponding naproxenamide derivatives 3a,b. Also, urea derivatives 5a,b were obtained through the reaction of compound 2 with sodium azide followed by reaction with 2-or 3-aminopyridene via Curtius rearrangement. In addition, naproxenoyl isothiocyanate was synthesized and reacted with 2- or 3-aminopyridine, where the thiourea derivatives 7a,b were obtained. Furthermore, cyanoacetanilide 9 was synthesized and reacted with acetylacetone, arylidenemalononitriles or arylidenecyanoacetate to furnish the corresponding pyridine amide derivatives 10-13. All compounds were screened for anti-inflammatory activity using an in vivo rat paw edema model, from which six of them exhibited higher potency than naproxen. The ulcerogenic effect of most of the active compounds was also screened. In ulcerogenicity screening, the potent anti-inflammatory compounds 3b, 5a and 5b were devoid of any ulcerative effect. This is contrary to naproxen which caused 100% ulcerative effect on all tested animals. Structure-based molecular modeling described that the virtual screening agrees with the SAR of in vivo anti-inflammatory and ulcerogenic activities. Furthermore, all the synthesized compounds were screened for their anti-microbial activity.

Keywords: Naproxen, Pyridine, Anti-inflammatory, Ulcerogenic, Antimicrobial activities, Surflex, Docking, COX-2, SYBYL-X

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INTRODUCTION

Currently available non-steroidal anti-inflammatory drugs (NSAIDs) (e.g. Ibuprofen, flurbiprofen, fenbufen and naproxen) exhibit gastro-intestinal toxicity.[1] Long-term use of these drugs has been associated with gastro-intestinal (GI) ulceration, bleeding and nephrotoxicity.[2] The GI damage from NSAIDs is generally attributed to decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining GI health and homeostasis[3,4] *via* non-selective inhibition of cyclooxygenases 1&2 (COX-1 & COX-2).[5] Coxibs (such as celecoxib, valdecoxib, rofecoxib, etoricoxib and lumiracoxib) are selective COX-2 inhibitors that were introduced two decades ago as the magic solution for the NSAIDs GI troubles.[6] Few years later, coxibs were implicated in cardiac side effects that led to withdrawal of rofecoxib and valdecoxib from the world markets.[7] The NSAIDs class, regardless their confirmed inhibition of COX-2, do not pose similar cardiac risk.[8, 9] Therefore, we turned to classic NSAIDs as convenient surrogates to discover new derivatives characterized by being potent anti-inflammatory but less ulcerogenic.[10] Our approach is to exploit certain differences between the COX-1 and COX-2 obtained from extensive structural biology studies in this subject.[11] For instance, the accessibility of the characteristic side pocket of COX-2 that accommodates one of the two pendent aromatic rings in coxib scaffolds and many similar selective COX-2 inhibitors. The isopropyl side chain of Val-523 was cited as the gate residue of this side pocket, but it leaves enough distance for penetration by a pendent aryl group in selective COX-2 inhibitors. In addition, the COX-2 active site is 25% larger and more flexible than that of hCOX-1. In COX-1, a bulkier isoleucine (Ile-523) blocks access to this pocket and the substrate binding domain is narrower and less flexible.[12]

We hypothesized that derivatization of the acidic group of naproxen into bulkier *N*-pyridyl carboxamides may lead to selective inhibition of COX-2 (Figure 1). The *N*-aryl group might be placed beyond Arg-120 (a residue near the mouth of the active site that interacts with naproxen's carboxylic group) and could be directed into the neighboring selectivity side pocket. In COX-1, this area does not tolerate bulky groups. Similar approach on hybrids of propyphenazone and antipyrine resulted in the enhancement of COX-2 selectivity.[13] Authors rationalized, through docking studies, that adding a bulky moiety to a non-selective COX inhibitor attenuates the COX-1 inhibition. Therefore, we decided to synthesize a series of amides of naproxen aiming to improve efficacy vs. toxicity profile.

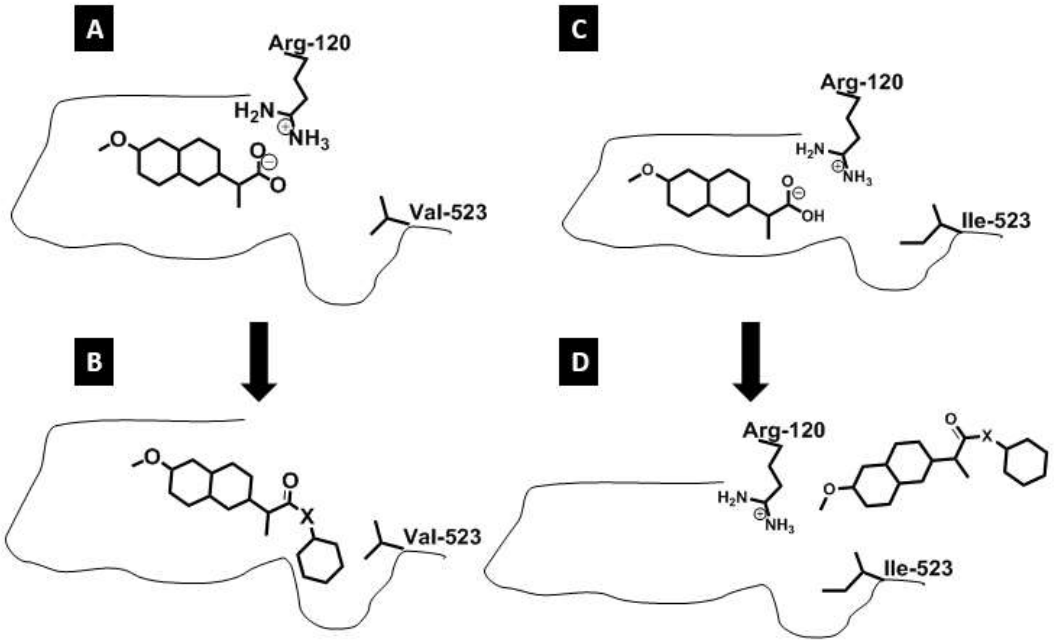


Figure 1. Rational design of naproxen derivatives as selective COX-2.

[A] Binding of naproxen to the active site of hCOX-2. Note that the selectivity side pocket is not attended by naproxen. [B] Designed naproxen derivatives have opportunity to invade the side pocket. [C] Binding of naproxen to the active site of hCOX-1. Note that the selectivity side pocket is closed by the bulkier Ile-523. [D] Designed naproxen derivatives are not able to stay in the active site of hCOX-1.

In addition to their anti-inflammatory uses, many members of the NSAIDs family possess antimicrobial activities. Moderate to remarkable antimicrobial activities of various compounds belonging to different pharmacological categories have been discovered. These categories include antihistamines,[14] antipsychotics,[15,16] and the anti-inflammatory agents. Many conditions describe inflammation occurring in response to a microbial infection. A combination of the anti-inflammatory drug along with a suitable antimicrobial agent is prescribed in such conditions. These combinations often cause side effects because of high drug doses. Searching for new compounds, that exhibit both anti-inflammatory as well as antimicrobial activities seems to be a interesting approach to overcome this problem. The search for antimicrobial activity among NSAIDs showed that diclofenac sodium exhibited significant potential antibacterial activity against both Gram-positive and Gram-negative bacteria. Piroxicam, mefenamic acid, naproxen and oxyphenbutazone were found to have mild to moderate antibacterial activities.[17] Furthermore, pyridine is the parent ring system of a large number of naturally occurring products and important pharmaceutical and agricultural chemicals.[18] In an attempt to discover new and useful agents for treating inflammatory diseases, we have replaced the carboxylic acid group of naproxen with an

additional pyridine moiety, which has been found to possess an interesting profile of anti-inflammatory activity with a significant reduction of the ulcerogenic effect. Structural modifications suggested in the present investigation focused mainly on studying the effect of linking various poly-substituted pyridines. The substitution pattern of the target compounds include various functionalities that would act as hydrogen-bond forming centers, such as carbonyl, amide, amino, cyano, hydroxy and carbethoxy groups. In addition, variation in the nature and size of other substituents was also attempted, as it would offer variable electronic, lipophilic and steric environments that would influence the targeted biological activity.

MATERIAL AND METHODS

Chemistry

All melting point were taken on Electrothermal LA9000 series, Digital Melting point Apparatus were uncorrected. IR Spectra were determined using KBr disc technique on Nikolet IR 200 FT IR Spectrophotometer at Pharmaceutical Analytical Unit, Faculty of Pharmacy, Al-Azhar University, and values are represented in (cm^{-1}). The ^{1}HNMR and ^{13}CNMR Spectra were recorded on Gemini 300MHz, and Mercury 400 MHz NMR Spectrometer at the Main Chemical Warfare Laboratories, Chemical Warfare Department, Ministry of Defense. DMSO-d_6 was used as a solvent; Chemical shifts were measured in δ ppm, relative to TMS as an internal standard. Mass Spectrum was recorded at 70ev on DI-50 unit of Schimadzu GC/ MS- QP5050A Spectrometer at Regional Center for Mycology and Biotechnology (RCMB), at Al-Azhar University represented as m/z (relative abundance %)(formula). Element Analysis (C, H, N) were carried out at Regional Center for Mycology and Biotechnology, Al-Azhar University, the values were found to be within± 0.4 % of the theoretical ones, unless otherwise indicated. Progress of the reaction was monitored by TLC using TLC sheets precoated with UV fluorescent silica gel Merck 60 F254 plates and was visualized using UV lamp.

2-(6-Methoxynaphthalen-2-yl)propanoyl chloride (2)

Synthesized according to reported method**.**[19]

Synthesis of 2-(6-methoxynaphthalen-2-yl)-*N*-(pyridine-yl)propanamide (3)

A mixture of **2** (0.01 mol), 2-aminopyridine (0.01 mol) and triethylamine (3 ml) in dioxane (30 ml) was refluxed for 3hr. The solution was treated with ice cold water. The obtained product was collected by filtration and recrystallized from ethanol.

2-(6-Methoxynaphthalen-2-yl)-*N*-(pyridine-2-yl)propanamide (3a)

White powder, **(**Yield 65%); mp 90-92 ^{o}C; IR: υ/cm^{-1}= 3204 (NH), 3058 CH-Ar), 2941, 2839

(CH-aliphatic), 1697 (C=O); ^{1}H NMR: δ/ppm = 1.48-1.50 (d, 3H, J=6.3 Hz CH_3), 3.85 (s, 3H, OCH_3), 4.14-4.16 (q, 1H, CH), 7.05-8.26 (m, 10H, Ar-H), 10.57 (s, 1H, NH exchangeable by D_2O); ^{13}C NMR; 18.76 (CH_3), 45.69 (CH), 55.59 (OCH_3), 113.85, 119.12, 119.81, 126.01, 126.85, 127.23, 127.28, 128.82, 128.85, 129.55, 129.60, 133.68, 133.71, 137.20, 138.57 (Ar-C), 173.68 (C=O); Mass spectrum exhibited a molecular ion peak at m/z: 307(M+1, 2.10%), 306 (M, 10.11%) with a base peak at m/z: 185 corresponding for (6-CH_3O-naphthylCHCH$_3$) ; Anal. Calc. for $C_{19}H_{18}N_2O_2$ (306.36): C, 74.49; H, 5.92; N, 9.14. Found: C, 74.62; H, 6.01; N, 9.27.

2-(6-Methoxynaphthalen-2-yl)-*N*-(pyridine-3-yl)propanamide (3b)

Pale yellow powder, **(**Yield 49%); mp 75-77 ^{o}C; IR: υ/cm^{-1}= 3362 (NH), 3049 (CH-Ar), 2984 (CH-aliphatic), 1674 (C=O); ^{1}HNMR: δ/ppm = 1.49-1.51 (d, 3H, J=6.9 Hz CH_3), 3.85 (s, 3H, OCH_3), 3.95-3.99 (q, 1H, CH),7.13-8.74 (m, 10H, Ar-H), 10.29 (s, 1H, NH exchangeable by D_2O). Mass spectrum exhibited a molecular ion peak at m/z: 307 (M+1, 2.66%), 306 (M, 10.55%) with a base peak at m/z: 185 corresponding for (6-CH_3O-naphthylCHCH$_3$). Anal. Calc. for $C_{19}H_{18}N_2O_2$ (306.36): C, 74.49; H, 5.92; N, 9.14. Found: C, 74.63; H, 6.01; N, 9.29.

2-(6-Methoxynaphthalen-2-yl)propanoyl azide (4)

Synthesized according to reported method**.**[19]

Synthesis of 1-(1-(6-methoxynaphthalen-2-yl)ethyl)-3-(pyridin-yl)urea (5)

A solution of **4** (0.01 mol) in benzene (30 ml) was heated under reflux for 2 hr. Aminopyridine (0.01 mol) was added and the mixture was refluxed for 2hr. On cooling, the precipitated solid was collected by filtration and recrystallized from ethanol.

1-(1-(6-Methoxynaphthalen-2-yl)ethyl)-3-(pyridin-2-yl)urea (5a)

White powder, Yield (64%); mp 165-167 ^{o}C; IR: υ/cm^{-1}= 3215 (NH), 3057 (CH-Ar), 2980, 2860 (CH-aliphatic), 1679 (C=O); ^{1}H NMR : δ/ppm = 1.48-1.51 (d, 3H, J=6.6 Hz CH_3), 3.85 (s, 3H, OCH_3), 4.98-5.01 (q, 1H, CH), 6.89-8.19 (m, 10H, Ar-H), 8.63, 9.16 (2s, 2H, 2NH exchangeable by D_2O); ^{13}C NMR; 23.52 (CH_3), 49.06 (CH), 55.59 (OCH_3), 106.18 ,112.08 ,117.29 ,119.12 ,124.13 ,125.61 ,127.44 ,128.73 ,129.64 ,133.76 ,138.65 ,140.37 ,147.28 ,153.90 ,154.42 (Ar-C), 157.51(C=O); Mass spectrum exhibited a molecular ion peak at m/z: 322 (M+1, 4.92%), 321 (M, 24.93%) with a base peak at m/z: 200**;** Anal. Calc. for $C_{19}H_{19}N_3O_2$ (321.37): C, 71.01; H, 5.96; N, 13.08. Found: C, 71.23; H, 6.02; N, 13.24.

1-(1-(6-Methoxynaphthalen-2-yl)ethyl)-3-(pyridin-3-yl)urea (5b)

Rose powder, Yield **(**80 %); mp 135-137 ^{o}C; IR: υ/cm^{-1}= 3306 (NH), 3054 (CH-Ar), 2971, 2860 (CH-aliphatic), 1621 (C=O); ^{1}HNMR: δ/ppm = 1.46-1.48 (d, 3H, J=6.6 Hz CH_3), 3.86 (s, 3H,

OCH_3), 4.94-4.98 (q, 1H, CH), 6.88 (s, H, -C(CH_3)-NH exchangeable by D_2O) 6.96-8.51 (m, 10H, Ar-H); 8.67 (s, H, -CO-NH exchangeable by D_2O); Mass spectrum exhibited a molecular ion peak at m/z: 322 (M+1, 1.12 %), 321 (M, 4.00%) with a base peak at m/z: 186**;** Anal. Calc. for $C_{19}H_{19}N_3O_2$ (321.37): C, 71.01; H, 5.96; N, 13.08. Found: C, 71.14; H, 6.01; N, 13.22.

Synthesis of 2-(6-methoxynaphthalen-2-yl)-*N*-(pyridine-ylcarbamothioyl)propanamide (7)

To a suspension of 2 in dioxane (30 ml), ammonium thiocyanate (0.01mol) was added. The reaction mixture was refluxed for 2 hr, cooled and filtered. The filtrate was treated with aminopyridine (0.01mol) and the mixture was refluxed for 3 hr, cooled and filtered. The solid product was crystallized from ethanol.

2-(6-Methoxynaphthalen-2-yl)-*N*-(pyridine-2-ylcarbamothioyl)propanamide (7a)

White powder, Yield (71%); mp.177-179 ^oC; IR: υ/cm^{-1}= 3344, 3180 (2NH), 3060 (CH-Ar), 2971, 2834 (CH-aliphatic), 1649 (C=O), 1395 (C=S); ^{1}HNMR: δ/ppm=1.36-1.39 (d, 3H, J=6.9 Hz, CH_3), 3.65-3.67 (q, 1H, CH), 3.85 (s, 3H, OCH_3), 6.18 (s, 2H, NH exchangeable by D_2O), 7.11-7.77 (m, 10H, Ar-H); ^{13}C NMR: 18.23 (CH_3), 45.29 (CH), 55.62 (OCH_3), 100.26, 106.11, 118.97, 119.23, 125.70, 126.59, 126.94, 127.39, 128.81, 129.65, 133.54, 133.68, 138.00, (Ar-C), 173.65 (C=O), 175.81 (C=S); Mass spectrum exhibited a molecular ion peak at m/z: 365 (M, 0.78%) with a base peak at m/z: 170; Anal. Calc. for $C_{20}H_{19}N_3O_2S$ (365.45): C, 65.73; H, 5.24; N, 11.50. Found: C, 65.89; H, 5.29; N, 11.63.

2-(6-Methoxynaphthalen-2-yl)-*N*-(pyridine-3-ylcarbamothioyl)propanamide (7b)

Pink powder, (Yield 75%); mp 115-117 ^oC; IR: υ/cm^{-1}= 3200 (NH), 3050 (CH-Ar), 2969 (CH-aliphatic), 1710 (C=O), 1388 (C=S); ^{1}H NMR: δ/ppm = 1.43-1.45 (d, 3H, J=7.2 Hz CH_3), 3.76-3.83 (q, 1H, CH), 3.86 (s, 3H, OCH_3), 7.12-7.77 (m, 10H, Ar-H), 7.80, 12.21 (2s, 2H, NH exchangeable by D_2O); Mass spectrum exhibited a molecular ion peak at m/z: 365 (M+1, 2.82%) 365 (M, 1.54%) with a base peak at m/z: 306; Anal. Calc**.** for $C_{20}H_{19}N_3O_2S$ (365.45): C, 65.73; H, 5.24; N, 11.50. Found: C, 65.88; H, 5.27; N, 11.63.

Synthesis of *N*-(4-(2-cyanoacetamido)phenyl)-2-(6-methoxynaphthalen-2yl)propanamide (9)

A mixture of **2** (0.01mol) in dioxane (20 ml) and *N*-(4-aminophenyl)-2-cyanoacetamide **(8)** (0.01mol) in *N*,*N*-dimethyl formamide (3 ml) was refluxed for 3 hr. The precipitate formed was collected by filtration and recrystallized from ethanol.

Deep violet powder Yield (45%); mp 265-267 ^oC; IR: υ/cm^{-1}= 3290 (NH), 3054 (CH-Ar.), 2941, 2850 (CH-aliphatic), 2259 (CH_2CN), 1662 (C=O); ^{1}HNMR: δ/ppm = 1.47-1.49 (d, 3H, J=6.9 Hz CH_3), 3.33 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.90-3.95 (q, 1H, CH), 7.12-7.80 (m, 10H, Ar-H),

10.03, 10.19 (2s, 2H, 2NH exchangeable by D_2O); Mass spectrum exhibited a molecular ion peak at m/z: 387 (M, 6.31%) with a base peak at m/z: 185 ; Anal. Calc. for $C_{23}H_{21}N_3O_3$ (387.43): C, 71.30; H, 5.46; N, 10.85. Found: C, 71.49; H, 5.53; N, 11.03.

Synthesis of *N*-(4-(3-cyano-4,6-dimethyl-2-oxopyridin-1(2*H*)-yl)phenyl)-2-(6-methoxynaphthalen-2-yl)propanamide (10)

To a solution of **9** (0.01mol) and acetyl acetone (0.01 mol) in ethanol (30 ml), piperidine (5 drops) was added. The mixture was refluxed for 3 hr, cooled and treated with ice cold water. The obtained product was collected by filtration and recrystallized from ethanol.

Pale violet powder Yield (51%); mp 235-237 ^oC; IR: υ/cm^{-1} = 3294 (NH), 3060 (CH-Ar.), 2956, 2850 (CH-aliphatic), 2223(C≡N), 1659 (2C=O); ^1H NMR: δ/ppm = 1.48-1.50 (d, 3H, J=6.9 Hz CH_3), 1.94, 236 (2s, 6H, $2CH_3$), 3.85 (s, 3H, OCH_3), 3.95-4.00 (q, 1H, CH), 6.39 (s, 1H, CH),7.12-7.80 (m, 10H, Ar-H), 10.02 (s, 1H, NH exchangeable by D_2O); Mass spectrum exhibited a molecular ion peak at m/z: 451 (M, 2.45%) with a base peak at m/z: 194. Anal. Calc. for $C_{28}H_{25}N_3O_3$ (451.52): C, 74.48; H, 5.58; N, 9.31. Found: C, 74.62; H, 5.69; N, 9.47.

Synthesis of 3-(aryl)-2-cyano-*N*-(4-(2-(6-methoxynaphthalen-2-yl)propanamido)-phenyl)-3-acrylamide derivatives (11a-c)

A mixture of **9** (0.01mol), the requisite aromatic aldehydes (0.01mol) in ethanol (30 ml) and piperidine (5 drops) was refluxed for 3 hr, cooled and treated with ice cold water. The obtained product was collected by filtration and recrystallized from ethanol.

3-(4-Chlorophenyl)-2-cyano-*N*-(4-(2-(6-methoxynaphthalen-2-yl)propanamido)phenyl)acrylamide (11a)

Yellowish brown powder Yield (66 %); mp 250-252 ^oC; IR: υ/cm^{-1}=3292 (NH), 3048 (CH-Ar.), 2949, 2850 (CH-aliphatic), 2218 (CN), 1653 (C=O); ^1H NMR δ/ppm = 1.49-1.50 (d, 3H, J=6.3 Hz CH_3), 3.85 (s, 3H, OCH_3), 3.94-3.96 (q, 1H, CH),7.12-7.99 (m, 14H, Ar-H), 10.09, 10.32 (2s, 2H, 2NH exchangeable by D_2O); Mass spectrum exhibited a molecular ion peak at m/z: 509 (M, 0.12%) with a base peak at m/z: 185; Anal. Calc. for $C_{30}H_{24}ClN_3O_3$ (509.98): C, 70.65; H, 4.74; N, 8.24. Found: C, 70.81; H, 4.78; N, 8.41.

2-Cyano-3-(2,4-dichlorophenyl)-*N*-(4-(2-(6-methoxynaphthalen-2-yl)propanamido)phenyl)acrylamide (11b)

Yellowish green powder Yield (65%); mp 180-182 ^oC; IR: υ/cm^{-1}= 3288 (NH), 3056 (CH-Ar), 2942 (CH-aliphatic), 2208 (CN), 1660 (C=O); ^1H NMR δ/ppm = 1.48-1.50 (d, 3H, J=6.6 Hz, CH_3), 3.85 (s, 3H, OCH_3), 3.92-3.96 (q, 1H, CH), 7.12-8.87 (m, 13H, Ar-H), 10.06, 10.41 (2s, 2H,

2NH exchangeable by D_2O); Mass spectrum exhibited a molecular ion peak at m/z: 544 (M, 7.07%) with a base peak at m/z: 44 ; Anal. Calc. for $C_{30}H_{23}Cl_2N_3O_3$ (544.43): C, 66.18; H, 4.26; N, 7.72 Found: C, 66.41; H, 4.32; N, 7.89.

2-Cyano-*N*-(4-(2-(6-methoxynaphthalen-2-yl)propanamido)phenyl)-3-(4-methoxyphenyl)acrylamide (11c)

Pale green powder Yield (83 %); mp 245-247 ^oC; IR: υ/cm^{-1}=3428, 3320 (2NH), 3021(CH-Ar.), 2820 (CH-aliphatic), 2219 (CN), 1660 (C=O); ^{1}HNMR δ/ppm= 1.48-1.51 (d, 3H, J=6.9 Hz, CH_3), 3.86 (s, 6H, $2OCH_3$), 3.99-4.05 (q, 1H, CH),7.12-8.29 (m, 14H, Ar-H), 10.20, 10.26 (2s, 2H, 2NH exchangeable by D_2O); Mass spectrum exhibited a molecular ion peak at m/z: 506 (M+1, 1.19%), 505 (M, 6.00%) with a base peak at m/z: 185; Anal. Calc. for $C_{31}H_{27}N_3O_4$ (505.56): C, 73.65; H, 5.38; N, 8.31 Found: C, 73.89; H, 5.47; N, 8.42.

Synthesis of *N*-(4-(6-amino-4-(aryl)-3,5-dicyano-2-oxopyridin-1(2*H*)-yl)phenyl-2-(6-methoxynaphthal-en-2-yl)-propanamides (12a-c)

To a solution of **9** (0.01 mol) and the arylidenemalononitriles (0.01 mol) in ethanol (30 ml), piperidine (5 drops) was added. The mixture was refluxed for 3 hr, cooled and treated with ice cold water. The obtained product was collected by filtration and recrystallized from ethanol.

***N*-(4-(6-Amino-4-(4-chloropyenyl)-3,5-dicyano-2-oxopyridin-1(2*H*)-yl)phenyl-2-(6-methoxynaphthalen -2-yl)propanamide (12a)**

Deep brown crystals Yield (57 %); mp 125-127 ^oC; IR: υ/cm^{-1} = 3427, 3316 (NH_2, NH), 3020 (CH-Ar), 2860 (CH-aliphatic), 2220 (CN), 1661 (C=O); ^{1}HNMR δ/ppm = 1.47-1.49 (d, 3H, J=6.6 Hz CH_3), 3.86 (s, 3H, OCH_3), 3.96-4.01 (q, 1H, CH), 7.16-7.95 (m, 10H, Ar-H), 9.65 (s, 1H, NH exchangeable by D_2O), 10.34 (s, 1H, NH_2 exchangeable); Mass spectrum exhibited a molecular ion peak at m/z: 576 (M+2, 9.88%), 574 (M, 12.51%) with a base peak at m/z: 135; Anal. Calc. for $C_{33}H_{24}ClN_5O_3$ (574.03): C, 69.05; H, 4.21; N, 12.20. Found: C, 69.34; H, 4.27; N, 12.37.

***N*-(4-(6-amino-3,5-dicyano-4-(2,4-dichlorophenyl)-2-oxopyridin-1(2*H*)-yl)phenyl-2-(6-methoxynaphth-alen-2-yl)propanamide (12b)**

Deep green powder Yield (55 %); mp 220-220 ^oC; IR: υ/cm^{-1} = 3297, 3154 (NH_2, NH), 3,060 (CH-Ar.), 2946 (CH-aliphatic), 2215 (CN), 1656 (C=O); ^{1}HNMR δ/ppm = 1.47-1.49 (d, 3H, J=6.6 Hz CH_3), 3.85 (s, 3H, OCH_3), 3.92-3.94 (q, 1H, CH),7.12-7.95 (m, 13H, Ar-H), 10.05 (s, 1H, NH exchangeable by D_2O), 10.19 (s, 2H, NH_2 exchangeable); Mass spectrum exhibited a molecular ion peak at m/z: 610 (M+2, 2.59%) with a base peak at m/z:48; Anal. Calc. for $C_{33}H_{23}Cl_2N_5O_3$ (608.47): C, 65.14; H, 3.81; N, 11.51. Found: C, 65.27; H, 3.78; N, 11.64.

***N*-(4-(6-amino-3,5-dicyano-4-(4-methoxyphenyl)-2-oxopyridin-1(2*H*)-yl)phenyl-2-(6-methoxynaphth-alen-2-yl)propanamide (12c)**

Gray powder Yield (71 %); mp 235-237 ^{o}C; IR: υ/cm^{-1} = 3294, 3160 (NH_2, NH), 3060 (CH-Ar.), 2951, 2850 (CH-aliphatic), 2212 (CN), 1659 (C=O); ^{1}HNMR δ/ppm = 1.47-1.50 (d, 3H, J=6.6 Hz CH_3), 3.83, 3.85 (2s, 6H, 2OCH_3), 3.92-3.95 (q, 1H, CH), 7.09-8.16 (m, 14H, Ar-H), 10.04 (s, 1H, NH exchangeable by D_2O), 10.18 (s, 2H, NH_2 exchangeable); ^{13}C NMR; 19.07 (CH_3), 46.25 (CH), 55.59 (2OCH_3), 115.32, 116.39 (2CN), 106.13, 119.09, 119.89, 120.04 ,120.10, 125.81, 126.76, 127.21, 128.82, 129.57, 130.24, 133.66, 134.14, 135.71 ,137.46, 157.50, 154.92, 161.05 (Ar-C)), 164.01, 172.53 (2C=O); Mass spectrum exhibited a molecular ion peak at m/z: 569 (M, 0.02%) with a base peak at m/z: 185 ; Anal. Calc. for $C_{34}H_{27}N_5O_4$ (569.61) : C, 71.69; H, 4.78; N, 12.30. Found: C, 71.78; H, 4.89; N, 12.48.

Synthesis of *N*-(4-(3,5-dicyano-6-hydroxy-4-(4-methoxyphenyl)-2-oxopyridin-1(2*H*)-yl)phenyl)-2-(6-methoxynaphthalen-2-yl)propanamide (13)

A mixture of **9** (0.01 mol), 4-methoxy ethyl cyanocinnamate (0.01 mol) and piperidine (5 drops) in ethanol (30 ml) was refluxed for 3 hr, cooled and treated with ice cold water. The obtained product was collected by filtration and recrystallized from ethanol.

Deep green powder; Yield (71 %); mp 230-232 ^{o}C; IR: υ/cm^{-1}= 3288 (br. OH, NH), 3057 (CH-Ar.), 2969, 2860 (CH-aliphatic), 2217(C≡N), 1658 (C=O); ^{1}H NMR δ/ppm = 1.47-1.49 (d, 3H, J=6.6 Hz CH_3), 3.85, 378 (2s, 6H, 2OCH_3), 4.29-4.31 (q, 1H, CH),7.14-8.31 (m, 14H, Ar-H), 10.01 (s, 1H, NH exchangeable by D_2O), 10.18 (s, 1H, OH exchangeable by D_2O) ; ^{13}C NMR; 14.47 (CH_3), 46.25 (CH), 55.59 , 56.09 (2OCH_3), 115.32, 115.43 (2CN), 106.13, 116.39, 119.10, 119.89, 120.04, 120.10, 121.43, 124.42, 125.81, 126.76 ,127.21, 128.82, 129.57, 132.96, 133.66, 133.97, 134.51, 135.72, 137.46, 154.92 ,157.50, 161.05 (Ar-C)), 164.01, 172.53 (C=O); Mass spectrum exhibited a molecular ion peak at m/z: 570 (M, 0.22%) with a base peak at m/z: 84 ; Anal. Calc. for $C_{34}H_{26}N_4O_5$ (570.79): C, 71.57; H, 4.59; N, 9.82. Found: C, 71.82; H, 4.66; N, 9.97.

Biological Activities

Anti-inflammatory activity

All the newly synthesized pyridine-containing compounds were assessed for their anti-inflammatory activity using carrageenan-induced hind paw edema method according to *Winter et al.* by employing 1% carrageenan solution as a phlogistic agent.[20] Edema was induced in the left hind paw of adult male albino rats weighing (150-200 g), obtained from the animal house

laboratory of Nile Company, Cairo, Egypt by the sub-planter injection of 0.1ml of 1% carrageenan in normal saline. Each group was composed of six animals. The equipment used was Dial micrometer model (120-1206 Baty, Sussex, England).

The animals which were bred in our laboratory were housed under standard conditions and received a diet of commercial food pellets and water *ad libitum* during maintenance, but were entirely fasted during the experiment period.

The test compounds were given orally 1 hr after carrageenan injection. Naproxen was taken as a standard at a dose of 30 mg/kg body weight orally.[21] The rat paw volume was measured after 1hr, 2hr, 3hr, 4hr and 5hrs respectively after carrageenan injection by using a plethysmometer. The size of edema was expressed as the increase in the thickness in mm after carrageenan injection.

Statistical analysis

The experimental data were presented as (Mean ± SEM) statistically analyzed using One Way Analysis of Variance (ANOVA) followed by Turkey compare test.

For the tested compounds **3a-12c**, the percent of edema inhibition after 1-5 hrs are presented in table **(1).**

Ulcerogenicity

All of the tested pyridine-containing compounds exhibiting an anti-inflammatory activity **3a-13c** were evaluated for their ulcerative effect on rats.

All animals subjected to this experimental test were sacrificed immediately after the last measurement (5 hrs) in a carbon dioxide chamber. The stomach was removed and opened along the greater curvature, washed with distilled water, and cleaned gently by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass. The number of ulcers was noted and the severity recorded with following scores.[22]

Scores 0= no ulcers; 1= superficial ulcers; 2=deep ulcers; 3=perforation

The ulcer index was expressed as (Mean ±SEM).

The percentage ulceration for each group was presented in table 2:

Antimicrobial testing

All of the newly synthesized compounds were evaluated for their antimicrobial activity using the agar diffusion technique; 1 mg/ml of the test compounds solution in dimethylsulfoxide was used. The tested organisms were *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 9372) as representatives of Gram positive bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 29853) as representatives of Gram negative bacteria and

Candida albicans (ATCC 10231) as representative of fungi. The bacteria and fungi were maintained on nutrient agar medium and Czapeks Dox agar medium, respectively. Dimethyl sulfoxide (DMSO) showed no inhibition zones. The agar media were inoculated with different test microorganisms. After 24 hours of incubation at 37 °C for bacteria and 48 hours of incubation at 28 °C for fungi, the diameter of inhibition zones (mm) were measured.

Culture Media:

For bacteria: Mueller Hinton Agar.

For Fungi: Sabouraud Dextrose Agar medium.

Methods

Antimicrobial tests were carried out using the agar well diffusion method. After the media had cooled down and solidified, wells (6 mm in diameter) were made in the solidified agar. The microbial inoculum was then uniformly spread using a sterile cotton swab on a sterile Petri dish containing Mueller Hinton agar (MHA) for bacteria and Sabouraud dextrose agar (SDA) for fungi, respectively. 100 µl of the tested compound solution was prepared by dissolving 1mg of the chemical compound in 1 ml dimethyl sulfoxide (DMSO). The inoculated plates were then incubated for 24 h at 37°C for bacteria and yeast and 48 h at 28°C for fungi. Negative controls were prepared using DMSO employed for dissolving the tested compound. Ampicillin (100 µg/ml), ciprofloxacin (100 µg/ml) and nystatin (100 µg/ ml) were used as standards for Gram-positive bacteria, Gram-negative bacteria and fungi respectively. After incubation, antimicrobial activity was evaluated by measuring the zone of inhibition against tested microorganisms. Antimicrobial activity was expressed as inhibition diameter zones in millimeters (mm).[23-25]

The obtained results were presented in (Table 3). The organisms were tested against the activity of concentration of 1mg/ml of the samples.

Minimum Inhibitory Concentration (MIC) measurement:

The MIC was determined by the broth microdilution method using 96-well micro-plates.[26, 27] The inoculate of the microbial strains was prepared from 24 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Each sample (5.0 mg) was dissolved in DMSO (1 ml) to obtain 500 µg/ml stock solutions. A number of wells were reserved in each plate for positive and negative controls. Sterile broth (100 µl) was added to the well from row B to H. The stock solutions of samples (500 µl) were added to the wells in rows A and B. The mixture of samples and sterile broth (100 µl) in row B was then transferred to each well in order to obtain a twofold serial dilution of the stock samples (concentration of 500, 250, 125, 62.5, 31.3, 15.6 and

7.81,3.9,1.95, 0.98 and 0.49 µg/ml). The inoculums (100 µl) were added to each well and a final volume 200 µl was obtained in each well. Plates were incubated at 37°C for 24 hrs in case of antibacterial activity and 48 hrs at 25°C for antifungal activity. Microbial growth was indicated by the presence of turbidity and a pellet at the bottom of the well. The results are shown in the following table (Table 4).

Molecular Modeling

The computer-assisted modeling was performed using SYBYL-X v. 2.1 software (Celera Corp., USA) licensed to King Abdulaziz University, Jeddah, Saudi Arabia. The crystal structures of enzymes involved in the study were downloaded from the Protein Databank website www.rcsb.org; Codes: human COX-2, 3OLU and human COX-1, 2OYU.[28] The protein structure were prepared according to Biopolymer Preparation protocol of SYBYL-X. In summary, the Biopolymer Preparation include the following steps: Remove unnecessary ligands present as a result of the crystallization process and remove iron of the heme; Add Hydrogen: H-Bonding; Termini Treatment: Charged; Add Charges: Appropriate charges were added for ionic residues; Staged Minimization: MMFF94s force field, MMF94 charges, Dielectric Constant: constant. The rest of energy minimization parameters were set default according to SYBYL-X sequence: Minimize Biopolymer Hydrogens; Minimize Water, Minimize Sidechains; Minimize Biopolymer with C-alpha; Minimize Ligands; Minimize All.

All compounds tested for anti-inflammatory activity were included in the study as well as naproxen as a reference drug. The molecular database of ligands were sketched on Chemsketch (ACD Labs) free software and saved as sdf file. The 2D sketches were converted to 3D structure with optimized geometry using Concord method and saved as SLN file.

Docking was performed using Surflex Program impeded in SYBYL-X.[29, 30] The docking mode was adjusted to Surflex-Dock Screen (SFXC). The protomol was generated around the extracted ligand of the corresponding crystal structure. The protocol included removal of all water and unnecessary ligands as recommended by Surflex. The protomol was carefully checked by visual inspection before proceeding to docking execution. Analysis of the results were performed according to given ranking scores listed in table 4.

RESULTS AND DISCUSSION

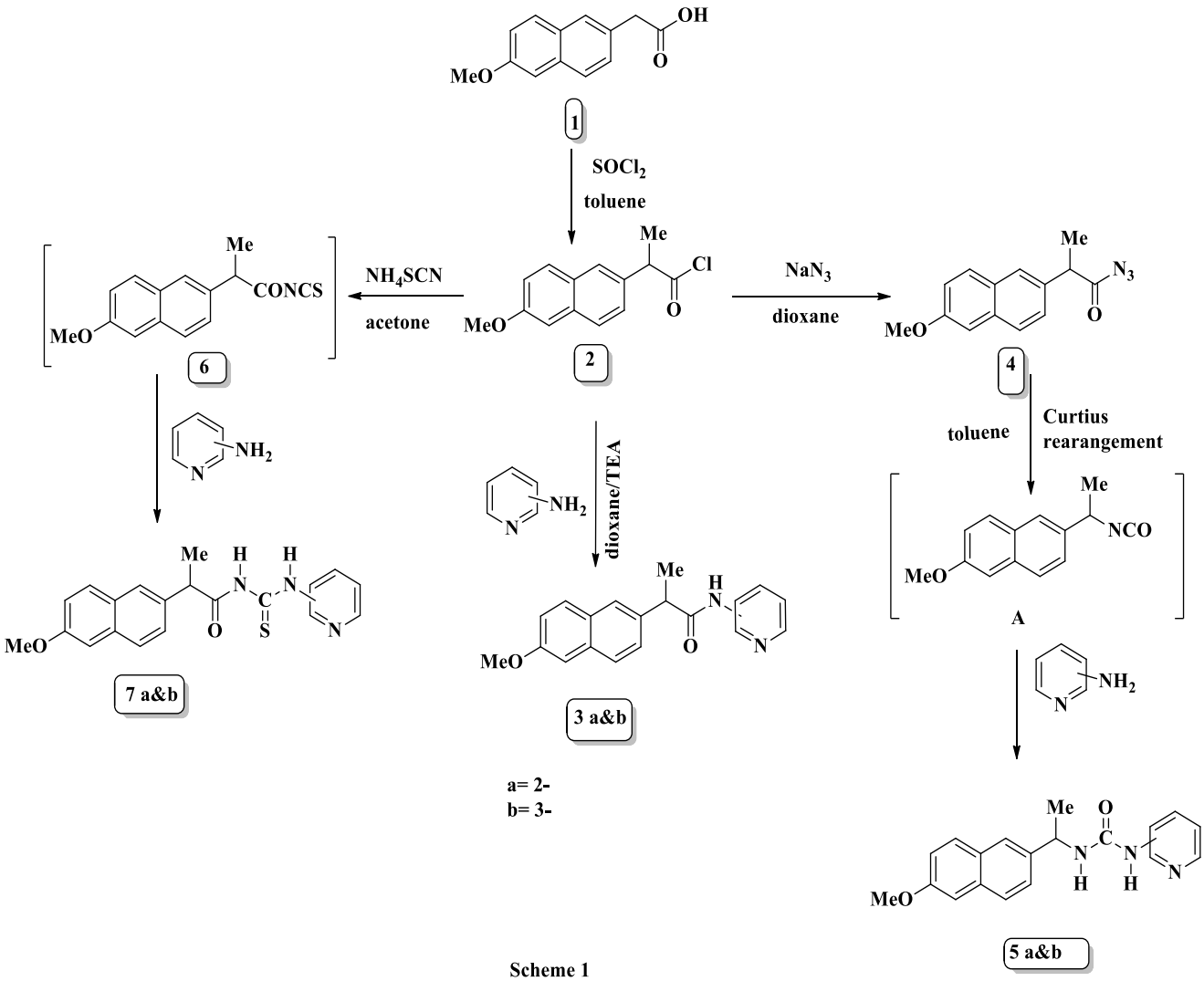
Chemistry

The synthesis of the target compounds is depicted in Schemes 1&2. The starting material 2-(6-methoxy-2-naphthyl)propanoic acid chloride **(2)** was obtained by refluxing of naproxen **(1)** with

thionyl chloride in toluene. Treatment of the acid chloride **(2)** with each of 2-or 3-aminopyridine in refluxing dioxane containing a catalytic amount of triethylamine afforded the respective pyridinecarboxamides **3a, b**. the structure of **3** has been assigned as a reaction product on the basis of spectroscopic data as well as elemental analysis. For example, the IR spectrum of compound **3a** revealed the absorption bands at 3204 cm^{-1} corresponding to NH function and 1697 cm^{-1} for C=O group.

^{1}HNMR spectrum for compound **3a** showed doublet and quartet at 1.48 and 4.14 specific for CH_3 and CH respectively, a singlet signal at .3.85 assignable to OCH_3 group, a multiplet signal at 7.05-8.62 region owing to aromatic protons and two singlet signals at 8.62 and 10.57 due to CH-pyridyl and NH protons. Its mass spectrum showed a molecular ion peak at m/z =306 corresponding to the molecular formula $C_{19}H_{18}N_2O_2$, with a base peak at m/z 185 corresponding to the (6-methoxynaphthalen-CH_3CH) group.

The novel urea derivatives **5a, b** were prepared from the reaction of the naproxen acid chloride with sodium azide, followed by the addition of aminopyridines. The formation of **5** is assumed to proceed *via* Curtius rearrangement of acid azide **4** into isocyanate intermediate (**A)** which subjected to aminopyridine addition scheme 1**.** The structure of the isolated compounds was achieved by its spectroscopic and elemental analysis. Thus, IR spectrum of **5a** showed a absorption band at 3215, 3306 cm^{-1} which indicates the presence of 2NH groups in addition to the presence of C=O and C=N at 1679, 1621 cm^{-1} respectively. Furthermore, ^{1}HNMR spectrum proved the presence of two singlet signals at 8.63 and 9.16 corresponding for 2NH groups exchangeable by D_2O. Also, ^{13}CNMR indicates the presence of C=O at 157.51 ppm. In addition, the mass spectrums indicate the presence of a molecular ion peak at m/z: 321 (M^+ 24.93%) corresponding to molecular formula $C_{19}H_{19}N_3O_2$ with a base peak at m/z 200. Moreover, the reaction of acid chloride **2** with ammonium thiocyanate in acetone under reflux gave naproxenoyl isothiocyanate **(6)**, which subjected for the reaction with 2-or 3-aminopyridine to furnish in each case only a sole product that was identified as *N*^{1}-naproxenoyl-*N*^{2}-pyridyl thiourea derivative **(7a,b)**. The structure of **7** was assigned on the basis of analytical and spectral data. The IR spectrum of **7a**, for example, showed absorption bands at 3344, 3180 cm^{-1} corresponding to NH functions, at 1649 cm^{-1} for C=O and at 1395 cm^{-1} for C=S. ^{13}CNMR of **7a** revealed the presence of C=O and C=S groups at 173.65 and 175.81. The mass spectrum showed a molecular ion peak (M+) at m/z = 365, corresponding to a molecular formula $C_{20}H_{19}N_3O_2S$.

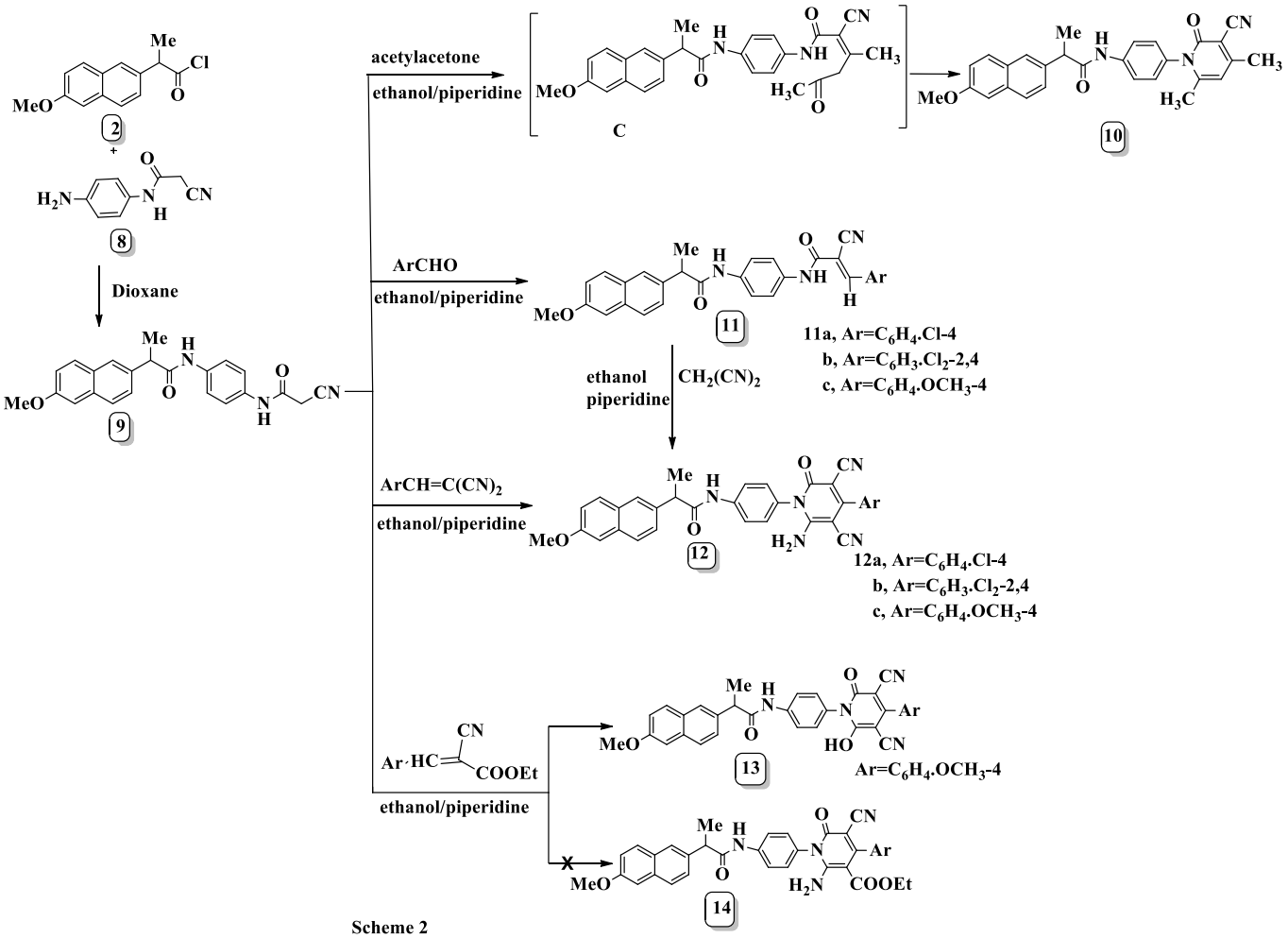


Scheme 1

Structure modifications of the carboxylic group in naproxen also extended to attain pyridinecarboxamides through another synthetic route. Thus, interaction of the naproxenoyl chloride **(2)** with *N*-(4-aminophenyl)-2-cyanoactamide **(8)** furnished *N*–[4-(cyanoacetamido)phenyl-2-(6-methoxynaphthalen-yl)propanamide **(9)** which is a deep violet powder.

Both elemental analysis and spectral data are in agreement with the proposed structure. Its IR analysis data indicates the presence of an absorption band at 3290 cm^{-1} representable for NH, in addition to a band at 2259 cm^{-1} for $CNCH_2$ and 1662 cm^{-1} for C=O. ^{1}HNMR showed singlet signals at 10.03 and 10.19 ppm for 2NH exchangeable by D_2O and a singlet signal at 3.33 due to a CH_2 group. Its mass spectrum exhibited a molecular ion peak at m/z= 387 (6.31%) corresponding to the molecular formula $C_{23}H_{21}N_3O_3$ with a base peak at m/z=185. This compound was used as a starting material for the synthesis of many pyridine-amide derivatives. When the cyanoacetanilide **9** was reacted with acetylacetone in the presence of a catalytic amount of piperidine,

cyclocondensation reaction occurred, and the 2-pyridinone derivative **10** was smoothly afforded. It can be postulated that the reaction initially proceeds *via* a nucleophilic attack to form Michael adduct **C** which in turn cyclized and eliminated a water molecule affording the final product. Structure of **10** was confirmed by spectral data and elemental analyses. IR spectrum indicates the presence of NH, CN and C=O groups at 3294, 2223 and 1659 cm^{-1} respectively. ^{1}HNMR showed, besides the naproxenoyl protons, two singlet signals were observed at 1.94 and 2.36 corresponding for two $2CH_3$, and an exchangeable signal at 10.02 ppm corresponding to an NH group. Moreover, the mass spectrum exhibited a molecular ion peak at m/z: 451 (M, 2.45%) corresponding to $C_{28}H_{25}N_3O_3$. In addition, condensation of the cyanoacetanilide **9** with aromatic aldehydes furnished the corresponding arylidene derivatives **11a–c.** Pyridin-2(1*H*)-one derivatives **12a–c** were obtained through the reaction of the arylidene derivatives **11a–c** with malononitrile in ethanol containing piperidine as catalyst. On the other hand, the 2-pyridone derivatives **12 a-c** were also obtained *via* reaction of cyanoacetanilide **9** with arylidene malononitrile upon heating under reflux in the presence of piperidine as catalyst (Scheme 2). The structure of compounds **11** and **12** were assigned on the basis of the elemental analysis and spectral data. IR of compound **12c** showed absorption bands at 3294, 3160, 2212 and 1659 attributed to NH_2, NH, CN, and CO groups respectively. The ^{1}HNMR spectrum (DMSO-d_6) revealed two singlet signals at 3.83 and 3.85 assignable to two OCH_3 and two singlet signals 10.04 and 10.18 due to NH and NH_2 protons. Also, ^{13}C NMR showed signals 115.32, 116.39164.01 and 172.53 due two 2CN and 2C=O groups respectively. The mass spectrum showed a molecular ion peak (M+) at m/z = 569 corresponding to the molecular formula $C_{34}H_{27}N_5O_4$. Finally, reaction of **9** with ethyl 4-methoxycyanocinnamate in ethanol containing a catalytic amount of piperidine afforded a single product for which two products **13** and **14** seemed possible. However, the hydroxypyridone derivative **13** was assigned for the reaction product on the basis of its elemental analysis and spectral data. The IR spectrum lacked an absorption band due to ester group and revealed absorption bands at 2217, 1658 for CN and CO groups. ^{1}HNMR showed a singlet signal at 10.18 ppm for exchangeable NH and a singlet at 10.18 for exchangeable OH. ^{13}C NMR also indicate the presence of $2OCH_3$ at 56.09 and 2CN at 115.32, 115.43, in addition to C=O at 172.53 ppm. Mass spectrum exhibited a molecular ion peak at m/z: 570 with a base peak at m/z: 84 (Scheme 2).



Scheme 2

Biological activity

Anti-inflammatory activity

All of the newly synthesized compounds containing pyridineamide-based appended naproxen moiety were tested for their *in vivo* anti-inflammatory activity using carrageenan induced rat hind paw edema method. Data of anti-inflammatory activity was expressed as mean ± SEM. The anti-inflammatory activity of the newly synthesized compounds **3a, 3b, 5a, 5b, 7a, 7b, 10, 12a, 12b, 12c and 13** in 30mg/kg were compared with the standard naproxen 30 mg/kg body weight. The results (Table 1, Figures 2) indicated that compounds **3b-10** possess significant anti-inflammatory activity. Amongst these compounds, compounds **5b and 7a** showed better activity (32.03%) at the first hour. The effect of **5b** was increased at the 3rd and 4th hours by 34.60% and 34.38% respectively. At the 5th hour, the effect was still greater than the first hour (33.12%) but less than the 3rd and 4th hours. This indicates that the drug has a rapid onset and its effect continues for the 5th hour when it begins to decline, although it remains more active than naproxen (25.93% at 5th

hour). The effect of naproxen as an anti-inflammatory drug begins low and reaches its maximum effect at the 3rd and 4th hours. The effect then begins to decline at the 5th hour. On the other hand, the anti-inflammatory effect of compound **7a** has a rapid onset and a long duration, where its effect increases to reach the maximum at the 5th hour (40.03%). Compounds **3b, 5a, 7b and 10** also showed significant anti-inflammatory activity (22.50%, 21.90%, 25.50% and 21.24%) respectively, comparable to the standard drug, naproxen, at 1st hour of inflammation (11.77%) but higher than that of control which showed 0.0% inhibition at all hours. At the 2nd hour, the compounds that showed the best effect compared to naproxen were **3a-10** which have (29.54%, 21.64%, 24.86%, 21.64%, 31.06%, 21.93% and 20.18%) respectively. Furthermore at the 3rd hour the most active compounds are **5a, b** and **7a, b,** which possess (38.89%, 34.60%, 39.65% and 35.87%). Moreover compound **3a-7b** with % of inhibition (38.84%, 38.34%, 38.33%, 34.38%, 39.58%, and 38.84%). Other compounds at this time entry were significant but their effect was less than the anti-inflammatory effect of naproxen. The effect of naproxen at the 5th hour decreased (25.93%) but the new active drugs still have high activity such as **3a-7b** which have (33.67%, 33.67%, 35.61%, 33.12%, 40.03%, 33.12%) % of inhibition. Compounds **10-13** have significant activity, but their effect is less than the effect of naproxen. From the previous discussion, it was concluded that the most active compounds are those that contain urea, thiourea and the pyridine derivatives **9**. Also their effect lasts for the last hour. This may be due to that the non-substituted pyridineamide-containing naproxen moiety compounds have a higher activity than the substituted one with an aryl group. This also may be due to the bulkiness of the compounds, where smaller compounds are more active than bulkier ones.

Table 1. Percent of edema inhibition for tested compounds

Compd. No.	1 h		2 h		3 h		4 h		5 h		
	Mean ± S.E.	% inh.	Mean ± S.E.	% inh.	Mean ± S.E.	% inh.	Mean ± S.E.	% inh.	Mean ± S.E.	% inh.	potency
Control	5.100± 0.057	_	5.750± 0.217	_	6.617± 0.289	_	6.733± 0.142	_	6.033± 0.044	_	_
Naproxen	4.500 ± 0.288	11.77	5.000± 0.086	12.29	4.533± 0.289 [a]	**31.32**	4.633± 0.289 [a]	31.16	4.467± 0.344 [a]	25.93	1
3a	5.083± 0.033	0.33	4.017± 0.016 [a,b]	**29.54**	4.400± 0.301 [a]	33.43	4.117± 0.033 [a]	**38.84**	4.000± 0.028 [a]	**33.67**	1.29
3b	3.950± 0.057 [a]	**22.50**	4.467± 0.235 [a]	**21.64**	4.600± 0.125 [a]	30.31	4.150± 0.100 [a]	**38.34**	4.000± 0.028 [a]	**33.67**	1.29
5a	3.983± 0.033 [a]	**21.90**	4.283± 0.120 [a]	**24.86**	4.033± 0.016 [a]	**38.89**	4.083± 0.016 [a]	**38.33**	3.883± 0.066 [a]	**35.61**	1.37

5b	3.467± 0.248 [a,b]	**32.03**	4.467± 0.248 [a]	**21.64**	4.317± 0.088 [a]	**34.60**	4.417± 0.233 [a]	**34.38**	4.033± 0.016 [a]	**33.12**	1.27
7a	3.467± 0.349 [a,b]	**32.03**	3.900± 0.125 [a,b]	**31.06**	3.983± 0.066 [a]	**39.65**	4.067± 0.044 [a]	**39.58**	3.617± 0.284 [a,b]	**40.03**	1.54
7b	3.800± 0.144 [a]	**25.50**	4.450± 0.236 [a]	**21.93**	4.233± 0.060 [a]	**35.87**	4.117± 0.044 [a]	**38.84**	4.033± 0.033 [a]	**33.12**	1.27
10	4.017± 0.016 [a]	**21.24**	4.550± 0.152 [a]	**20.18**	4.917± 0.033 [a]	25.51	4.867± 0.159 [a]	27.69	4.617± 0.284 [a]	23.44	0.90
12a	4.983± 0.016	2.29	5.033± 0.016	11.70	5.017± 0.016 [a]	24.00	5.033± 0.016 [a]	25.22	4.950± 0.057 [a]	17.92	0.69
12b	5.025± 0.034	0.41	5.183± 0.083	9.07	5.067± 0.044 [a]	23.24	4.967± 0.016 [a]	26.20	5.067± 0.016 [a]	15.98	0.61
12c	4.667± 0.308	8.50	5.033± 0.016	11.7	5.050± 0.000 [a]	23.49	5.033± 0.016 [a]	25.22	5.033± 0.016 [a]	16.54	0.63
13	5.083± 0.033	0.33	5.200± 0.104	8.78	4.817± 0.233 [a]	27.03	5.033± 0.016 [a]	25.22	5.050± 0.028 [a]	16.26	0.62

[a] means significantly different from control value at P<0.05. [b] means significantly different from naproxen value at P<0.05 . Values represent the mean ± standard error of means (SEM) of six animals for each group.

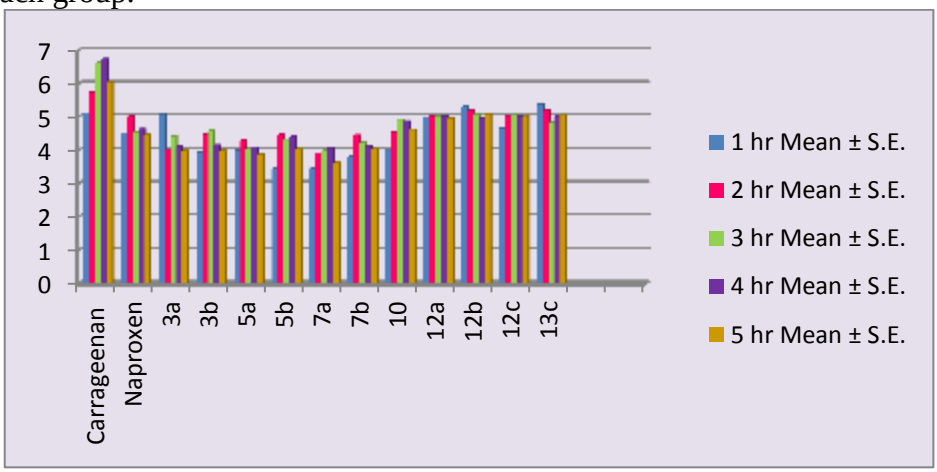
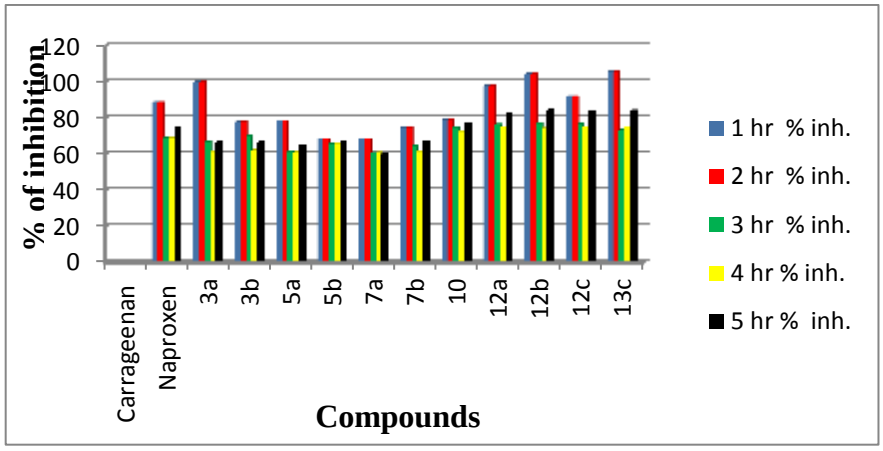


Figure 2. (A) Mean ± S.E. at 1st, 2nd, 3 rd, 4th &5th hrs.



(B) Percentage of Inhibition at 1st, 2nd, 3 rd, 4th &5th hrs.

Ulcerogenicity

In general, it was found from the above table that the most of the tested compounds showed better results than the reference drug, naproxen. Especially, compounds **3b,5a,5b,10,12a,b,c** were devoid of any ulcerative effect compared to a 100% ulcerative effect of naproxen as illustrated in the previous Table 2 and Figure 3.

Table 2. The percentage ulceration

The percentage ulceration					
No of compd.	**Mean ± SEM**	**%of inhibition**	**No of compd.**	**Mean ± SEM**	**%of inhibition**
Control	0.00±0.00***	0.00	**7b**	1.333±0.333**	36.36
Naproxen	3.667±0.333	100	**10**	0.00±0.00***	0.00
3a	1.667±0.333**	45.45	**12a**	0.00±0.00***	0.00
3b	0.00±0.00***	0.00	**12b**	0.00±0.00***	0.00
5a	0.00±0.00***	0.00	**12c**	0.00±0.00***	0.00
5b	0.00±0.00***	0.00	**13**	1.333±0.333**	36.36
7a	1.667±0.333**	45.45	*** indicate the drug is more significant 3 times than Naproxen. ** indicate the drug is more significant 2 times than Naproxen.		

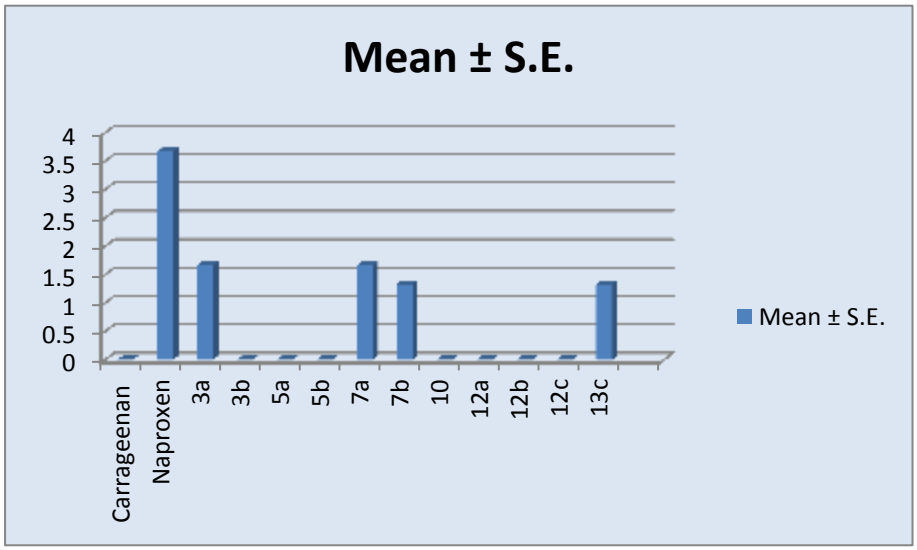
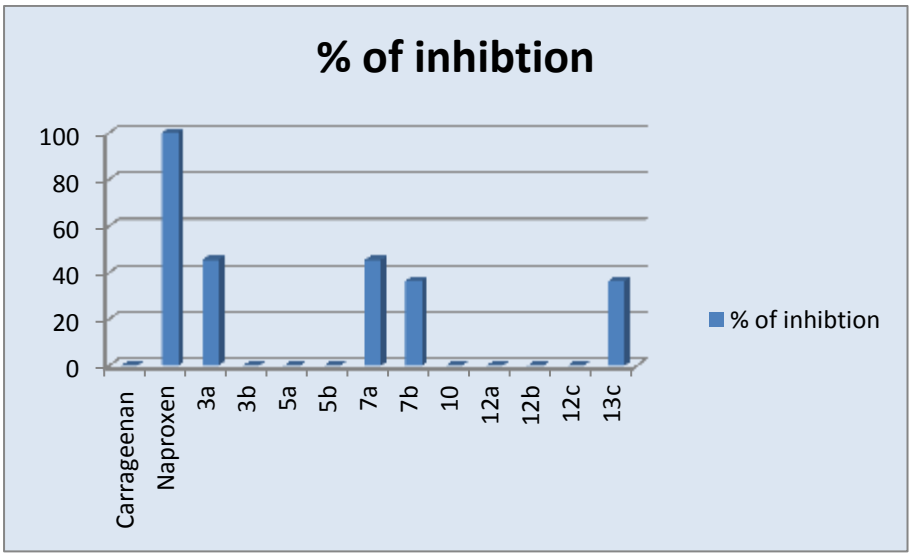


Figure 3. (A) Mean ± S.E. of ulcer effect.



(B) Percentage of inhibition of ulcer effect.

Antibacterial activity

All the newly synthesized compounds were tested *in vitro* for their antibacterial activity against the following bacterial strains: *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 9372) as representatives of Gram-positive bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 29853) as representatives of Gram-negative bacteria and *Candida albicans (ATCC 10231)* as a representative of fungi. The results are summarized in Table 3. Antimicrobial tests were carried out by the agar well diffusion method using a solution of the tested compound prepared by dissolving 1 mg of the chemical compound in 1 ml of dimethyl sulfoxide (DMSO) as shown before. After incubation time, antimicrobial activity was evaluated by measuring the inhibition zone diameters against the test organisms and compared with standard zone size ranges that determine susceptibility, intermediate susceptibility, or resistance to the screened compounds. The inhibition zone diameters show that most tested compounds in the antibacterial assay possess significant activity against the growth of most test organisms especially *C. albicans* in comparison with the naproxen and the standard drugs used. It was shown that all compounds have activity against *B. subtilis* which is a Gram-positive bacterium, while the standard drug was inactive against this strain of bacteria. In a comparison with naproxen, it was found that compounds number **3a, 10, 11c, 12a** and **12b** showed higher activity than it. Only **2** compounds **7a&b** were found to have antibacterial activity against *S. aureus* in comparison to standard and naproxen. This may be due to the presence of pyridine ring attached to NH-CS-NH moiety. Moreover, it was found that most tested compounds as well as naproxen have moderate activity against Gram-negative bacteria such as *E. coli* and *P. aeruginosa* than ciprofloxacin. On the other

hand, all tested compounds, as well as naproxen, except **3b** were shown to possess high activity against *C. albicans* rather than the standard nystatin.

Table 3. Diameter of zone of inhibition in mm (*In vitro* activity against)

Compd. no.	Diameter of zone of inhibition in mm				
	Gram +Ve		Gram –Ve		Fungi
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***
Naproxen	22	--	10	12	**35**
3a	26	--	9	17	**36**
3b	20	--	12	7	**--**
5a	12	--	11	10	**31**
5b	23	--	7	11	**22**
7a	14	8	9	6	**33**
7b	12	3	9	11	**26**
10	25	--	11	--	**27**
11a	22	--	10	--	**27**
11b	23	--	14	--	**25**
11c	24	--	10	--	**26**
12a	25	--	11	--	**25**
12b	24	--	--	--	**27**
12c	21	--	10	11	**26**
13	22	--	10	15	**21**
Standard	--	--	24	24	14

Standard= Ampicillin for Gram-positive bacteria, ciprofloxacin for Gram-negative bacteria and nystatin as antifungal.

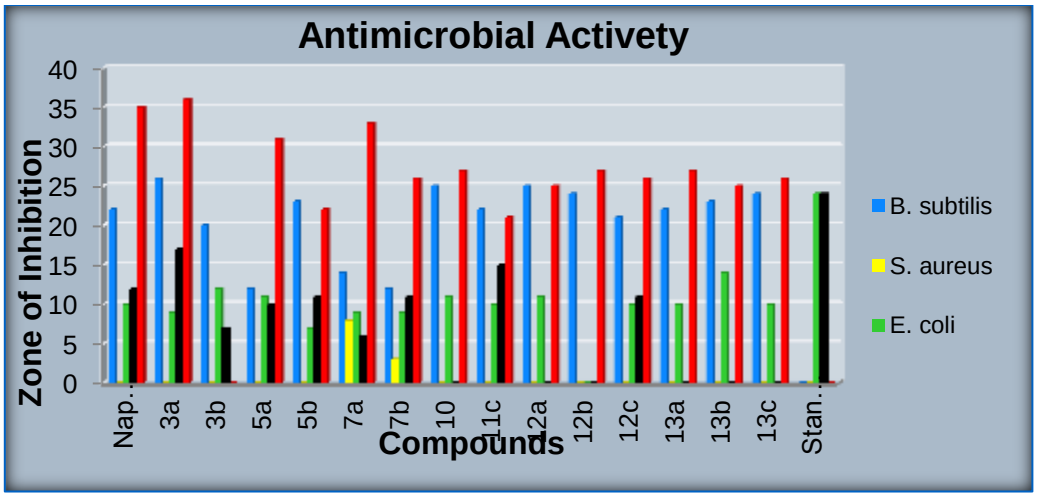


Figure 4. Diameter of zone of inhibition in mm.

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) is a standard technique used to measure the lowest concentration of a compound required to inhibit the visible growth of a microorganism being investigated after approximately 24 hrs of incubation in the appropriate growth medium. MIC of

active compounds was studied against *B. subtilis, S. aureus E. coli, P. aureginosa, and C. albicans*. The results obtained from the test against *B.subtilis* antimicrobial sensitivity indicated that naproxen, **3a, 5b** and **12c** active compounds exhibited antimicrobial activity with concentrations of 15.6, 3.9, 3.9 and 15.6 μg/ml respectively. Moreover, the results obtained of *S .aureus* showed high resistance for all active compounds except compound **7b** which inhibited the growth of *S .aureus* at a concentration of 250 µg/ ml. Also, the results obtained in the table of tested organism *E. Coli* antimicrobial sensitivity indicated that naproxen, **3b, 5a, 11a, 11b, 11c, 12c and 13** are active compounds that exhibit an antimicrobial activity at concentrations 250, 31.3, 31.3, 15.6, 15.6, 15.6, 31.3 and 31.3 µg/ml respectively. On the other hand, the results obtained in table 4 of tested organism *P. aureginosa* antimicrobial sensitivity indicated that naproxen, **3a, 5b, 7b and 12c** are active compounds that exhibit an antimicrobial activity at concentrations 250, 15.6, 250, 250 and 250 μg/ ml respectively. Finally, the results of *C. albicans* antimicrobial sensitivity showed that it is highly sensitive for all active compounds except compounds **3b, 5b** and **11a** which developed resistance of *C. albicans*. (Table 4 and Figure 5)

Table 4. Minimum Inhibitory Concentration (MIC) of the active compounds in µg/ ml.

Minimum inhibitory concentration in µg/ ml					
	Test organisms				
Compd.	Gram +Ve		Gram -Ve		Fungi
No.	***B.subtilis***	***S.aureus***	***E.coli***	***P.aureginosa***	***C.albicans***
Naproxen	15.6	Nt	250	250	15.6
3a	3.9	Nt	Nt	15.6	3.9
3b	Nt	Nt	31.3	Nt	Nt
5a	Nt	Nt	31.3	Nt	15.6
5b	3.9	Nt	Nt	250	Nt
7b	Nt	250	Nt	250	31.3
11a	Nt	Nt	15.6	Nt	Nt
11b	Nt	Nt	15.6	Nt	31.3
11c	Nt	Nt	15.6	Nt	31.3
12a	Nt	Nt	Nt	Nt	15.6
12b	15.6	Nt	Nt	Nt	31.3
12c	Nt	Nt	31.3	250	15.6
13	Nt	Nt	31.3	Nt	31.3

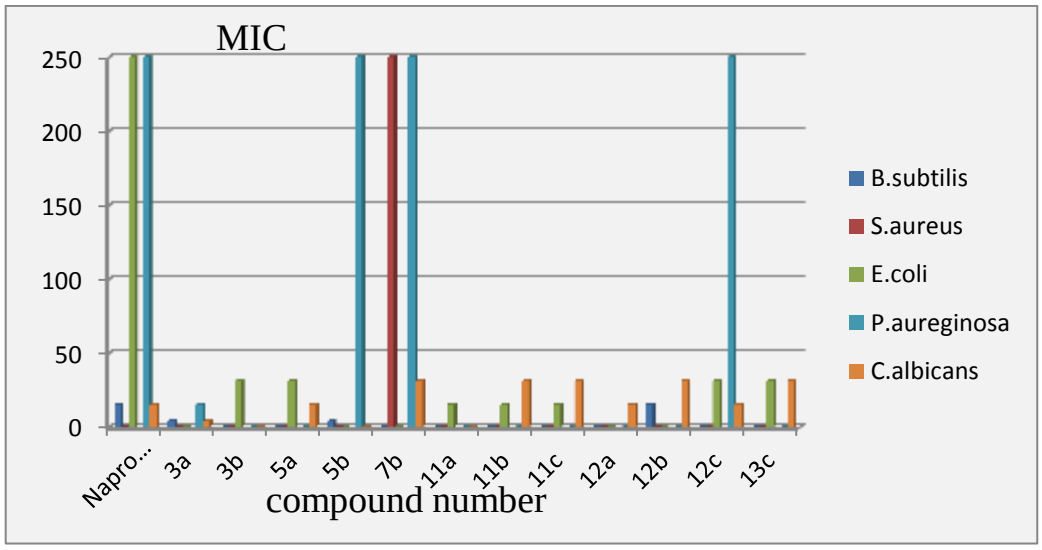


Figure 5. Minimum Inhibitory Concentration (MIC) of the active compounds in µg/ ml.

Structure-Based Molecular Modeling

The docking experiments were employed to rationalize the high anti-inflammatory potency. The crystal structure of COX-2 used in the study (PDB Code: 3OLU)[31] was selected based on certain criteria to get the most suitable conditions for Surflex to predict the binding model that is closest to reality. Indeed, Surflex binding predictability is affected by the ligand of the crystal structure without ignoring features of the active site as determined by Surflex probes. The COX-2 crystal structure contains a glyceryl ester of arachidonic acid (AG), a non acidic (ester) ligand in which Arg-120 interacts with 2'-hydroxy group of the glyceryl moiety *via* a hydrogen bond but not as ionic salt bridge as in case of the free acid.[32] It is analogous to our compounds which are devoid of carboxylic acid groups. The second advantage of having AG as a ligand is that it has a very long hydrophobic tail composed of 20 carbon atoms. The end of this hydrocarbon chain (the ω carbon) reaches to the bottom of the active site which is characterized by a strong hydrophobic environment. This is advantageous to our docking study because the modeled compounds are significantly bulkier than parent drugs. This agrees with solid evidence about the importance of hydrophobic interaction of COX-2 inhibitors with the hydrophobic channel of the active site.[31]

Surflex generally predicted binding ranks parallel to *in vivo* anti-inflammatory activity results (Table 5). Compounds **12a-c** and **13,** which bear very bulky biaryl substituents on the amide nitrogen, gave the lowest scores and the least anti-inflammatory response in edema test. Naproxen itself was not the best scoring compound as predicted by Surflex and observed by anti-inflammatory test. The highest scoring compound was the di-substituted urea **5b** which exhibited the second highest potency in the edema inhibition test.

Table 5. Scoring function of screened compounds as resulted from a docking experiments using Surflex program (default SYBYL-X settings).

Compound	hCOX-2 Score	hCOX-2 Rank	hCOX-1 Score	hCOX-1 Rank
5b	4.744	1	1.711	4
3b	4.246	2	2.813	3
3a	3.961	3	3.268	2
5a	3.893	4	1.395	5
7a	3.555	6	-0.323	7
7b	3.062	7	0.012	6
12c	-0.047	8	-13.537	11
12a	0.469	9	-9.254	10
12b	-2.372	10	-5.891	8
13	-3.613	11	-7.471	9
(S)-Naproxen	3.667	5	4.928	1

Analysis of the docking mode by visual inspection revealed that the compound binds conveniently in the active site as the naphthyl portion stays in the active site main channel and mostly show hydrophobic attractions. The compound passes through the wider polar mouth area and make two effective bifurcate hydrogen bonding interaction *via* the two NHs of the urea moiety with the backbone carbonyl of Val-523 (Figure 6B). The compound invades deep in the selectivity side pocket (Figure 6A) *via* insertion of its pyridyl moiety into the selectivity side pocket. The pyridyl group showed only hydrophobic interaction inside this pocket (*c.f.* celecoxib which make some hydrogen bonds at this site).(11) In figure 6C, the electrostatic potential around the ligand **5b** is mostly hydrophobic except a small area close to the urea moiety, confirming the complete dependence on both geometry and balanced architecture in possible binding with COX-2. Docking of such compounds into COX-1 crystal structure. The compound 7b, that showed the highest anti-inflammatory potency in edema screening test, was among the high scoring compounds but the binding mode, as predicted by Surflex, did not include effective hydrogen bonds. This is not a surprise because the *in vivo* results are usually not identical to *in silico* screening. The amidic compounds, **3a** and **3b,** were smaller than optimum and could not reach the side pocket in the active site.

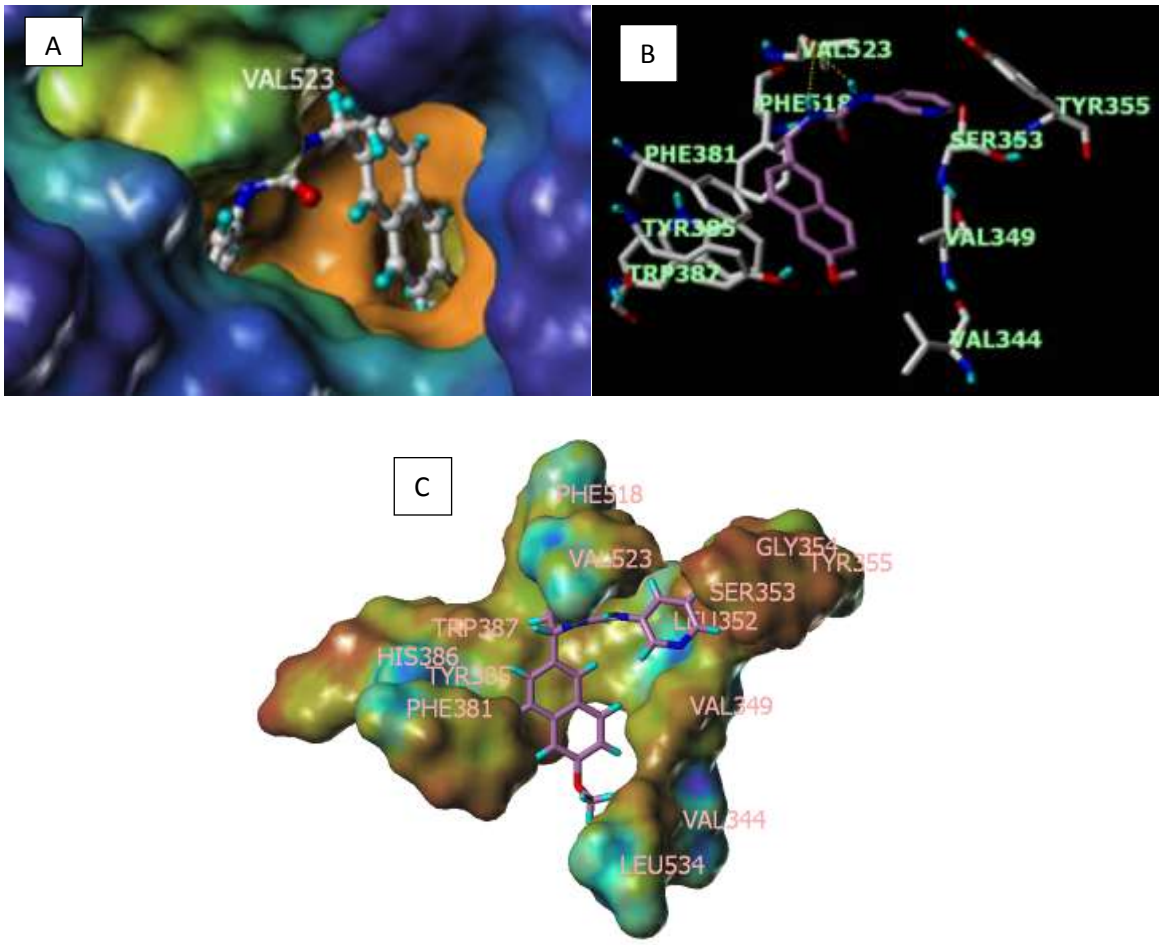


Figure 6. Prediction of binding mode of the top scoring compound 5b.

[A] Accommodation of the compound inside the active site. The protein was given a water accessible (Connolly) surface that is colored by cavity depth (blue, outer and brown deeper). The main active site gorge is to the right and the naphthyl ring goes deep inside it. The side selectivity pocket is shown to the left in which the pyridyl group is positioned. Note the appropriate distance and geometry of the urea group passing through the gate residue Val-523. The following residues were hidden from the display for clarity of the image: Pro191, Gln192; Leu-352 to Tyr355; Trp387 & Pro-514 to Phe518. [B] Residues interacting with the ligand 5b. The hydrogen bonds are given dashed yellow lines by SYBYL-X display. [C] All the residue of the active site interacting with ligand 5b. They were give Connolly surface colored by Poisson-Boltzmann electrostatic potential (Blue is most polar and brown is the least polar).

CONCLUSION

In this research, the derivatization of well-established drug structures as an approach to avoid known toxicity has delivered promising dual anti-inflammatory and antimicrobial leads. We confirmed that the replacement of the carboxylic head group of NSAIDs with another polar group such as amide or urea (hydrogen bonding group) that carry a substituent such as a heteroaryl ring retain the anti-inflammatory activity and diminished the GI irritation. Our structure-based

modeling attributed the high *in vivo* potency and low toxicity of certain compounds in the series (such as **5b)** to their selective inhibition of COX-2 enzyme over the COX-1 enzyme. The model suggests that the large hydrophobic moiety present in NSAIDs structure, when linked to non-acidic bridge that carry a moderate size heteroaryl group, can be considered for future design and development of safer anti-inflammatory drugs. The hydrophobic portion of NSAIDs, such as the 6-methoxynaphthyl of naproxen, for instance, fills the main hydrophobic gorge of the COX-2 active site while the polar bridge hooks to one of the polar residues in the mouth area of active site *via* hydrogen bonding. This architecture seemingly forces the heteroaryl moiety to be inserted in the selectivity side pocket to achieve better inhibition of COX-2 over COX-1.

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