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Design, synthesis, docking and evolution of fused imidazoles as anti-inflammatory and antibacterial agents

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ABSTRACT

*New benzimidazole and imidzopyridine derivatives were designed and synthesized from their corresponding oximes **II**. The newly synthesized compounds were investigated in vivo for their anti-inflammatory and analgesic activities. Some of the new compounds (**IVc, IVf, IVi**) showed reasonable anti-inflammatory and analgesic activity in experimental rats in comparing to indomethacin and Diclofenac Na, as reference drugs Moreover, some of the newly synthesized compounds tested as antimicrobial agents, **IVh** exhibited potent antimicrobial activities with low MIC in comparing with Cefoperazone and Fluconazole as reference drugs Docking studies was carried out for derivatives of highest anti-inflammatory activity into the COX-2 binding site. Designed compounds **IVc,IVf,IVi** revealed a similar binding mode to COX-2 inhibitor.*

Key words: Fused Imidazoles**,** Anti-inflammatory, Anti-microbial, Docking studies, COX-2 inhibitors

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INTRODUCTON

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the choice treatment in various inflammatory diseases such as arthritis, rheumatisms as well as to relieve the aches and pain of everybody life. [1] Classical NSAIDs exhibit their action by restricting the biosynthesis of prostaglandin, this is essentially brought about by inhibiting the cyclo- oxygenase (COX) enzyme which involved in the inflammatory cascade .[2] It is well known (NSAIDs) are associated with several side effects such as gastro-intestinal mucosal damage and renal toxicity. [3, 4] Therefore, development of novel compounds having anti-inflammatory and analgesic activity with an improved safety profile is still needed

The concomitant use of several drugs to treat inflammatory conditions that might be associated with some microbial infections may cause health problems especially in patient with impaired liver or kidney functions. In addition, from the pharmaco-economic point of view, and for seeking a better patinat compliance, an anti-inflammatory-antimicrobial agents with minimum adverse effects and high safety margin is highly desirable

Fused imidazole derivatives have occupied a prominent place in medicinal chemistry because of their significance properties as therapeutics in clinical application. [5-8] In particular, Benzimidazole and imidazopyridine pharmacophore have drawn a great attention due to their wide range of chemotherapeutic activities including antibacterial, [9] Antimicrobial, [10]Anti-inflammatory, [11,12]Anti-Cancer, [13-15] Anti parasitic ,[16]Antiviral ,[17]anti -diabetic,[18] Anti- histaminic ,[19] inhibitors of Phospho inositide 3-Kinase signaling ,[20] Anticoccidial agents ,[21] Antiprotozoal, [22] estrogen receptor modulators[23] and Anti HIV [24]

Thus our main objective is to design novel benzimidazole and imidazopyridine derivatives as anti-inflammatory-antimicrobial agents. Our strategy is focusing in using traditional medicinal chemistry techniques motivated by comparative modeling of COX-2 enzyme crystallized with Indomethacin. Computer docking technique plays an important role in drug design. We docked the designed compounds into COX-2 binding site with FlexX-Leadit-

2.1.2. [25] as flexible docking program that enables us to predict favorable protein-ligand complex structures and MOE 2010.10 [26]program was used for visualization the orientation of the docked compounds at the active site of the COX-2 enzyme.

MATERIALS AND METHODS

2.1 Chemistry

Fine chemicals were purchased from prolabo chemicals. All solvents & reagents used were of reagent grades and obtained from the local scientific distributors in Egypt. Melting points are uncorrected and were determined using an Electro thermal 9100 apparatus. Elemental analysis was performed at Services Laboratory, Cairo University. IR spectra were recorded on a Beckman infrared spectra photometer PU 7712 using KBr. NMR spectra were recorded on JEOL EX-270 MHz and JEOL ECA 500 MHz spectrometer. Using suitable deuterated solvent and TMS was used as an internal standard. The mass spectra were recorded on GCMS-QP 1000Ex, Shimadzu spectrometer E.I. 70 eV at the Central Services Laboratory, Cairo University and National Research Centre, Cairo, Egypt. (Samples were centrifuged using a Heraeus Labofuge 400R (Kendro Laboratory Products GmbH, Germany) and the spectro photometric measurements were recorded using Shimadzu UV-VIS Recording 2401 PC spectrophotometer. Japan .The well-known compounds, 2- *(*1H-benzimidazol-2-yl) acetonitrile **Ia,** 2-(6-bromo-3H-imidazo [4,5-b] pyridin-2-yl) acetonitrile **Ib,** 2-(1H-benzo[d] imidazol-2-yl)-2-(hydroxyimino) acetonitrile **IIa** and **IVa. [**27]were prepared following the procedures reported in the literature.

2.1.1. 2-(6-bromo-3H-imidazo [4,5-b] pyridin-2-yl)-2-(hydroxyimino) acetonitrile (IIb).

2-(6-bromo-3H-imidazo [4,5-b] pyridin-2-yl) acetonitrile **Ib** (0.01 mole, 2.3 g) in diluted HCL (10 ml) was treated with sodium nitrite solution (0.01 mole, 0.7 g in 2 ml water) portion wise with stirring at room temperature. After 10 minute the product started to separate out but stirring was maintained for further 30 minutes. The solution was then filtered off and crystallized to afforded **IIb** as yellow crystal, yield 70%, m.p >300 ºC (dioxin); IR υ: 2213(CN), 3261(NH), 3426(OH) cm^{-1};^{1}H-NMR(DMSO-d_6) δ: 9.9(S, H, NH imidazopyridine, exchangeable with D_2O), 12.27 (S, H, OH exchangeable with D_2O), 7.8-8.62 (m,2H,Ar -H), MS (m/z): 264.85[M^+](27.99%). Anal Calc for $C_8H_4BrN_5O$. Calcd: % C, 36.12; H, 1.52; N, 26.32. Found: %C, 36.11; H, 1.53; N, 26.33.

2.1.2. General procedure for the synthesis 3-(substitunt)-5-hydroxyimino pyrimido (1,6-a) benzimidazole-2-imino- 4-one (IVb-IVi)

A solution mixture of the compounds **IIa, b** (0.01 mole) and appropriate isocyante in dry pyridine (20ml) was refluxed for 12hr.The reaction mixture was concentrated, cooled and poured gradually onto iced water while stirring, the solid formed was filtered off, washed with dilute hydrochloric acid then with water, dried, crystallized from the appropriate solvent

2.1.2.1. 3-(p-Nitophenyl)-5-hydroxyiminopyrimido (1,6-a) benzimidazole-2-imino- 4-one (IVb)

White yellowish, yield 74%, m.p 303-305 ºC, (butanol); IR υ: 1664 (CO amide) 3368(OH), 3331 (NH) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 9.6(s, H, NH, exchangeable with D_2O), 11.7 (S, H.OH exchangeable with D_2O), 7.68-8.19 (m, 8H, Ar-H), MS (m/z): 350[M^+] (7%), Anal calc for $C_{16}H_{10}N_6O_4$: Calcd.: %C,54.86 ; H, 2.88 ; N,23.99 . Found: %C, 54.87; H, 2.89; N, 23.98.

2.1.2.2 3-(2-Methoxyphenyl)-5-hydroxyiminopyrimido (1,6-a) benzimidazole-2- Imino-4-one (IVC)

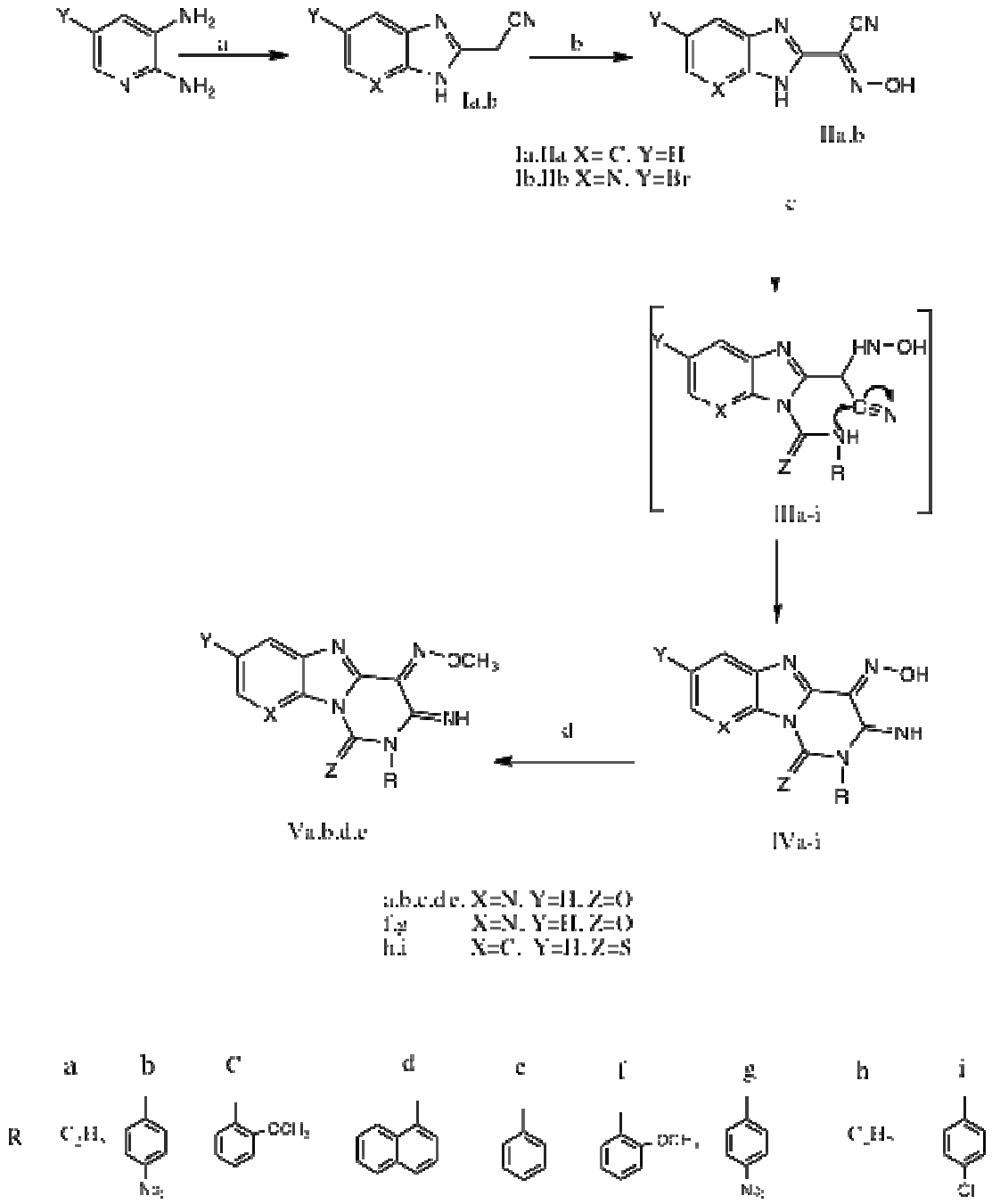
Brown, 78% yield, m.p 187-190 ºC, (methanol); IR υ: 1646 (CO amide) 3317(broad band NH, OH) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 8.8 (s, H, NH, exchangeable with D_2O), 11.9 (S, H.OH exchangeable with D_2O), 3.8 (S, 3H, OCH_3), 6.90-8.04 (m, 8H, Ar-H) MS (m/z): 335[M^+] (2%). Anal calc for $C_{17}H_{13}N_5O_3$, Calcd. %C, 60.89; H, 3.91; N, 20.89. Found: %C, 60.87; H, 3.90; N, 20.87.

2.1.2. 3 3-Naphthalene-5-hydroxyiminopyrimido (1,6-a) benzimidazole-2- imino-4-one (IVd)

Brown, 78% yield, m.p 295-297 ºC, (butanol); IR υ: 1627(CO amide), 3444 (OH), 3279(NH) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 8.01 (s, H, NH, exchangeable with D_2O), 11.1 (S, H.OH exchangeable with D_2O), 7.46-7.63 (m, 11H, Ar-H C-H), MS (m/z): 355[M^+] (4%). Anal calc for $C_{20}H_{13}N_5O_2$ Calcd. ; %C, 67.60; H, 3.69; N, 19.71. Found: %C, 67.62; H, 3.70; N, 19.72.

2.1.2.4 3-phenyl-5-hydroxyiminopyrimido (1,6-a) benzimidazole-2- imino-4-one (IVe)

Yield 75%, m.p 238-240 ºC. (butanol); IR υ: 1645(CO amide). 3326(OH), 3284(NH) cm^{-1}; 1H-NMR (DMSO-d_6) δ: 10.5 (broad band, NH, OH exchangeable with D_2O), 6.9- 7.4 (m, 9H, Ar-H), MS (m/z): 304[M^+ - 1] (5%), Anal calc for $C_{16}H_{11}N_5O_2$) Calcd. %C, 62.95; H, 3.63; N, 22.94. Found: %C, 62.99; H, 3.65; N, 22.97.



Reaction Condition: a; Ethylcyanoacetate , fusion 170 ℃, b; HCL , Sodium nitrite, c; isocyanate derivatives,dry pyridine, d; methyl iodide, potassium carbonate,dry DMF

Scheme 1

2.1.2. 5 3-Ethyl-5-hydroxyiminopyrimido (1, 6-a) benzimidazole-4-imino-2-thione (IVh)

Yellow, yield 78%, m.p >300 ºC, (butanol); IR υ: 3274(OH, NH broad band) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.4 (t.,3H,CH_3, ethyl group), 3.2 (q,2H,CH_2, ethyl group), 8.6 (S,H,NH, exchangeable with D_2O), 13.1 (S, H.OH exchangeable with D_2O), 7.21-7.62 (m,4H, Ar-H). MS (m/z) 273.31[M^+] (1.07%), Anal calc for $C_{12}H_{11}N_5OS$, Calcd: %C, 52.73; H, 4.06; N, 25.62. Found: %C, 52.78; H, 4.07; N, 25.64.

2.1.2.6 3-(*p*-Chloro Phenyl)-5-hydroxyiminopyrimido (1, 6-a) benzimidazole-4-imino-2-thione (IVi)

Yield 75%; m.p >300 ºC, (butanol); IR υ: 3423 (OH, NH broad band) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 9.8 (S, H, NH, exchangeable with D_2O), 10.8 (S, H.OH exchangeable with D_2O), 7.3-8.3 (m, 8H, Ar-H). MS (m/z,) 355[M^+] (0.8 %). Analysis for $C_{16}H_{10}ClN_5OS$, M.wt (355.8) Calcd; %C, 54.01; H, 2.83; N, 19.68. Found: %C, 54.04; H, 2.86; N, 19.70.

2.1.2.7 3-(2-methoxyphenyl)-6 -bromo-5-hydroxyiminopyrimido-imidazo (4, 5-b) pyridine-2- Imino-4-one (IVf)

Yellow , yield 78%, m.p>300ºC ,(ethanol); IR υ: 1671(CO amide) 3435(OH), 3328(NH) cm^{-1};^{1}H-NMR ($CDCL_3$-d_6) δ : 8.11 (s,H,NH, exchangeable with D_2O), 11.12 (S, H. OH exchangeable with D_2O), 3.8 (S, 3H, OCH_3), 6.9-8.1 (m, 6H, Ar-H), MS (m/z): 414.9[M^+-1] (17%). Anal calc for C_{16} $H_{11}BrN_6O_3$, Calcd %C, 46.28; H, 2.67; N, 20.24. Found: %C, 46.27; H, 2.68; N, 20.26.

2.1.2.8 3-(*p*-nitrophenyl)-6-bromo-5-hydroxyiminopyrimido-imidazo (4, 5-b) pyridine-2- Imino-4-one (IVg)

Yellow, yield 78%, m.p>300ºC, (ethanol); IR υ: 1646(CO amide) 3317 (broad band OH, NH) cm^{-1}; ^{1H}-NMR (DMSO-d_6) δ: 10.54 (s,H, NH, exchangeable with D_2O),11.58 (S, H.OH exchangeable with D_2O), 9.3-9.4 (m,6H, aromatic proton) , MS (m/z): 430 [M^+] (17%), Anal calc for C_{15} $H_8BrN_7O_4$, Calcd.: %C, 41.88 ; H, 1.87 ; N,22.79 .Found: %C, 41.887; H, 1.87; N, 22.79.

2.1.3. General procedure for the synthesis 3-(substituant)-5-methoxyimino pyrimido (1,6-a) benzimidazole-2-imino-4-one Va,Vb ,Vd,Ve.

A solution of methyl iodide (0.01 mole, 1.4g) was added to Compound **IVa,b** and **IVd,e** (o.o1 mole) in dry DMF (25 ml) in presence of 0.5 g of K_2CO_3 (0.5 ml), and refluxed for 6 hr, the reaction mixture was poured on iced water while stirring, precipitate was formed, filtrated off, dried and crystallized from the appropriate solvent

2.1.3.1 3-Ethyl-5-methoxyiminopyrimido (1,6-a) benzimidazole-2-imino- 4-one (Va)

Colorless, yield 75%, m.p >300 ºC, butanol /DMF (5:1) ; IR υ: 1600(CO amide).,3300 (NH) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 8.2 (s,H,NH, exchangeable with D_2O) 1.2 (t,3H,CH_3, ethyl group),3.50(q,2H,CH_2,ethyl group), 7.26-7.73 (m,4H, Ar-H)and 4.1 (S,3H,OCH_3),MS(m/z): 271.27[M^+] (10%), Anal calc for C_{13} $H_{13}N_5O_2$, Calcd; %C, 57.56 ; H, 4.83 ; N,25.82 . Found: %C, 57.53; H, 4.82; N, 25.81.

2.1.3.2 3-(p-nitophenyl)-5-methoxyiminopyrimido (1, 6-a) benzimidazole-2-imino-4-one (Vb.)

Brown, yield 72%, m.p 274-276 ºC, butanol /DMF (5:1); IR υ: 1600(CO amide), 3420(NH) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 8.2 (s, H, NH, exchangeable with D_2O), 4.1 (S,3H, OCH_3), 7.26-8.23 (m,8H, Ar-H) , MS (m/z): 364.09 [M^+] (1%), Anal calc for C_{17} $H_{12}N_6O_4$, Calcd.: %C, 56.05 ; H, 3.32 ; N,23.07 . Found: %C, 56.08; H, 3.34; N, 23.09,

2.1.3.3 3-Naphthalene-5-methoxyiminopyrimido (1, 6-a) benzimidazole-2-imino-4-one (Vd)

Colorless, yield 78%, m.p 264-266 ºC, butanol /DMF (5:1); IR υ: 1620(CO amide), 3420(NH) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 8 (s, H, NH, exchangeable with D_2O), 4.1(S, 3H. OCH_3), 6.96-7.73 (m, 11H, Ar-H), MS (m/z): 369 [M^+] (5%), Anal calc for $C_{21}H_{15}N_5O_2$, Calcd.: %C, 68.28 ; H, 4.09 ; N,18.96 . Found: %C, 68.26; H, 4.07; N, 18.94.

2.1.3.4 3-phenyl-5-methoxyiminopyrimido (1, 6-a) benzimidazole-4-imino- 2-one (Ve)

Colorless, yield 70%, m.p 246-248 ºC, (butanol); IR υ: 1600(CO amide) 3362(OH), 3420(NH) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 8.2 (s, H,NH, exchangeable with D_2O), 4.1 (S, 3H.OCH_3), 7.26-7.73 (m,9H, Ar -H), MS (m/z) : 319[M^+] (5%), Anal calc for $C_{17}H_{13}N_5O_2$, Calcd.: %C,63.94 ; H, 4.10 ; N,21.93 . Found: %C, 63.95; H, 4.12; N, 21.95.

2.2 Biological screening: -

Animals-adult rat of both sexes weighing 150-200 g and adult mice weighing 20-25 g were used in the experiments. Animals were housed under standardized conditions for light and temperature and received standard rat chow, tap water and libitum. Animals were randomly assigned to different experimental groups, each kept in a separate cage, All animal procedures were performed after approval from the Ethics committee of the National research Center and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No.85-23, revised 1985)

2.2.2. Anti-inflammatory activity screening:

All the synthesized compounds were tested for their anti-inflammatory activity using carrageenan induced rat hind paw edema method of Winter et al. [29]The edema hind paw was induced by injection of 0.1 ml of 1% carrageenan solution into sub-planter region of right hind paw. The volume of the paw was measured with a planimeter immediately and 2hour after the injection of the irritant. Tested compounds (100mg/kg b.w.) and indomethacin (50mg/kg b.w.) were injected (I.P), 1 hr before carrageenan injection. The mean value for each group was calculated and compared with control. Percent protection was calculated (Table 1, Fig. 1) using the following formula:(1-Vt/Vc)*100. Where Vt = Mean volume of edema in test animals and Vc= Mean Volume of oedema in control

Table 1 The anti-inflammatory activity of tested compounds

Compound	Paw oedema thickness (mm)			
	0 hr (X±SE)	% edema inhibition	2 hr (X±SE)	% edema Inhibition
Control	1.63±0.05	-	1.86±0.05	-
IVa	1.50±0.00	04.00	1.70±0.05	07.14
IVb	1.80±0.05	-	1.80±0.05	03.57
IVc	1.40±0.10	14.28	1.23±0.05	33.92
IVd	1.56±0.05	16.06	1.43±0.05	23.21
IVe	1.70±0.05	05.35	1.46±0.05	21.42
IVf	1.40±0.05	12.20	1.23±0.05	33.92
IVg	1.70±0.11	-	1.86±0.05	-
IVh	1.63±0.05	-	1.46±0.05	21.40
IVi	1.33±0.05	18.35	1.16±0.05	37.63
Va	1.60±0.05	-	2.20±0.11	-
Vb	1.73±0.05	-	2.10±0.05	-
Vd	1.86±0.05	-	1.86±0.05	-
Ve	1.86±0.05	-	2.13±0.05	-
Indomethacin	1.10±0.05	28.57	0.93±0.05	49.99

Data represent mean value ± SE

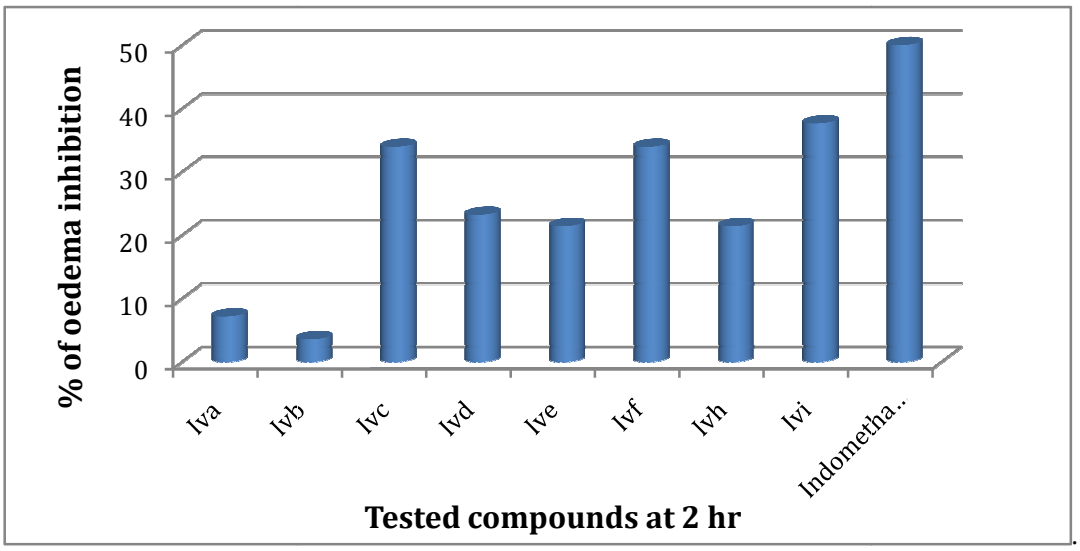


Fig. 1. The percentage of edema inhibition of the tested compounds and slandered drug Indomethacin

Table 2 Analgesic activity of tested compounds

Compound	Mean writhing (X±SE)	%Protection
Control	20.00±0.05	-
IVa	19.60±1.52	03.26
IVb	20.66±2.08	-
IVc	06.30±0.05	68.84
IVd	09.30±1.15	54.09
IVe	13.00±1.00	36.05
IVf	05.00±0.05	75.04
IVg	20.00±1.00	01.60
IVh	11.00±1.00	45.80
IVi	04.66±1.00	77.04
Va	21.33±1.52	-
Vb	21.00±1.52	-
Vd	19.00±1.00	06.54
Ve	18.30±0.05	09.82
Diclofenac Na	03.00±0.00	85.24

Data represent mean value ± SE

2.2.3. Analgesic activity screening: -

Acetic acid induced writhing model was used to evaluate analgesic activity of the synthesized compounds. Five groups of six Swiss albino mice were used.0.6% acetic acid (dose =10 ml/kg) was injected intra-peritoneal. The

numbers of writhes were counted for 20 min, after 5 min of injection of acetic acid into each mouse. This reading was taken as a control. Tested compounds (dose 100 mg/Kg), or Diclofenac Na (reference drug, dose 50 mg/Kg) were Injected I.P, 1 hr before injection of acetic acid. After 5 min of acetic acid injection, Mice were observed for the number of writhing for the duration of 20 minute. The mean value for each synthesized compounds and SE was calculated and Percent protection was calculated. (Table 2. Fig. 2)

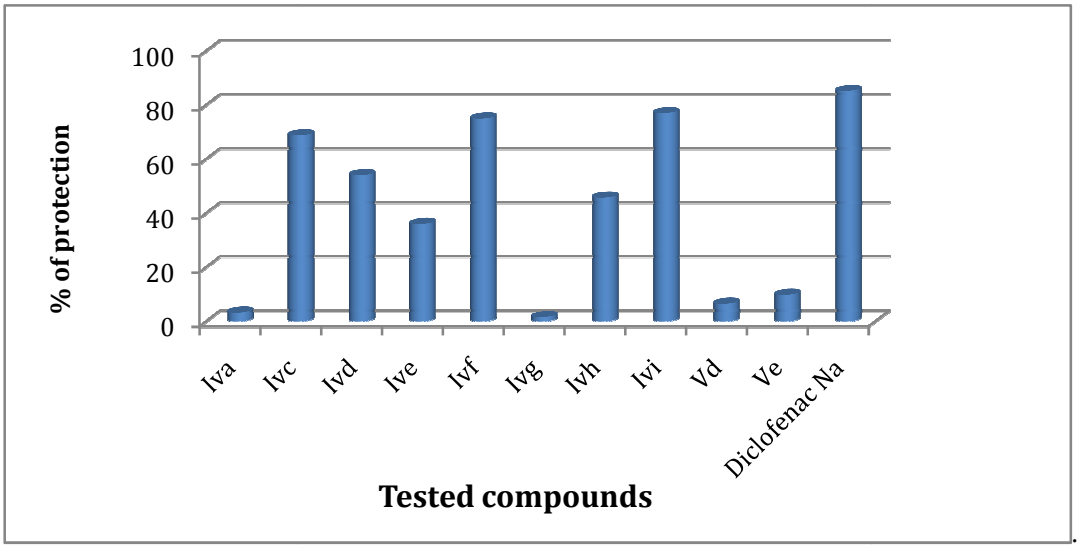


Fig. 2 Analgesic activity of tested compounds and standard drug Diclofenac Na

2.2.4. Antimicrobial screening: -

The tested compounds were evaluated in vitro against two Gram-negative bacterial strain E. coli, S. typhimurium, two Gram-positive bacterial strains: B.Cereus and S. aureus and two fungi strain C. albicans and A.flavus using paper disc diffusion technique. The sterilized medium.[30]was inoculated with the suspension of the microorganism and poured into Petri dish (3-4 mm depth). The paper impregnated with tested compounds which dissolved in DMSO, then placed on the solidified medium. The plates incubated at 37°C. Cefoperazone (75 ug/ml) and Fluconazole (20 ug disc) were used as standered for anti-bacterial and antifungal, respectively the observed inhibition zone is presented in (Table 3)

Table 3 Antimicrobial activities of tested compounds

Compounds	Inhibition zone diameter (mm/mg sample)					
	S. aureus	*B. cereus*	*E. coli*	*S. typhimurium*	*C, albicans*	*A. flavus*
IVc	10	9	11	10	-ve	-ve
IVd	14	12	13	13	-ve	-ve
IVe	12	10	-ve	-ve	-ve	-ve
IVf	-ve	-ve	-ve	13	-ve	
IVh	30(0.078)	27(0.039)	40(0.039)	36(0.039)	30(0.078)	30(0.039)
IVi	-ve	-ve	-ve	-ve	-ve	-ve
Va	-ve	-ve	-ve	-ve	-ve	-ve
Vb	-ve	-ve	-ve	-ve	-ve	-ve
Vd	-ve	-ve	-ve	-ve	-ve	-ve
Ve	-ve	-ve	-ve	-ve	-ve	-ve
Cefperazone	14(25)	14(25)	13(25)	12(25)	-ve	-ve
Fluconazole					14(12.5)	14(12.5)

Values in parenthesis are MIC in µg/ml

2.3. Molecular Docking methodology: -

All molecular modeling studies were performed on a RM Innovator Pentium IV2.4 GHz running Linux Fedora Core 3. The protein crystal structure was downloaded from (http://www.rcsb.org / -pdb code: 4COX). Hydrogen atoms were added to the protein, using the protentate 3D option in MOE 2010.10 (Molecular Operating Environment). (http://www.chemcomp.com.) Ligand structures were built with MOE and Energy minimized using the MMFF94x force field until a RMSD gradient of 0.05 Kcal mol-1 A°-1 was reached. Docking procedure was done using Flex-X-Leadit.2.1.2 program.

RESULTS AND DISCUSSION

3.1 Chemistry:

The designed target compounds were obtained as outlined in scheme 1. Starting with fusion of o-phenlyn diamine and / or 5-bromo-2, 3-diamino pyridine with ethylcyanoacetae provided compounds 2- (1H-benzimidazol-2-yl) acetonitrile **Ia** and (6-bromo-3H-imidazo [4,5-b] pyridin-2-yl) acetonitrile **Ib** in good yield. Treatment **Ia,b** with sodium nitrite in presence of diluted HCL afforded 2-(1H-benzo[d] imidazol-2-yl)-2-(hydroxyimino)acetonitrile **IIa**, and 2-(6-bromo-3H-imidazo [4,5-b] pyridin-2-yl)-2-(hydroxyimino) acetonitrile (**IIb**), as starting materials, the structure of these compounds was elucidated by elemental analysis as well as their spectral features and previous report . Reflux **IIa,b** for 12hr with isocyanate derivatives in dry pyridine resulted in 5-hydroxyiminopyrimido-benzimidazole(imidazopyridine)-2-imino- 4-one derivatives **IVa-i** via the formation of the intermediate Michael type products **IIIa-I,**.followed by intramolecular cyclization.[28] The spectral and microanalyical data for compounds **IVa-i** consistent with their chemical structures. For example The IR spectrum of **IVg** of imidazo pyridine derivatives, revealed the absence of the CN peak which appeared in the parent compound **IIb**, Moreover, the mass spectrum of **IVg** showed a molecular ion peak at m /z 430.6 which in accordance with its molecular formula In addition, **IVa,b, d,e** undergoes alkylation with methyl iodide in the presence of anhydrous potassium carbonate. Affording the **Va,b,d,e** in moderate yield, The IR spectrum of this series showed disappearance the OH group. Moreover, the 1H-NMR showed the disappearance the oximino OH proton appeared in the parent compounds **IVa,b,d,e**, and showed a new signal corresponding to the -OCH3 group at δ 4.1 ppm.

3.2 Biological activity

2.2.1. In vivo anti-inflammatory studies

The carrageenan-induced rat paw oedema bioassay was used for compounds **IVa-IVi and Va,b,Vd.e.** The results are summarized in (Table 1, Fig. 1). Compound **IVi** showed excellent protection against inflammation (37.63%) and the two analogues **IVc,IVf** exhibited reasonable reduction in oedema size with a percent of inhibition (33.92%).while **IVd** ,**IVe** and **IVh** exhibited moderate anti-inflammatory activity, (23.21%,21.42%,21.40%) in comparison with indomethacin which has percent inhibition (49.99%).The rest of the compounds exhibited weak to non anti-inflammatory activity.

From the structure activity relationship (SAR) viewpoint, the pyrimidine ring with N-aromatic substitution derivatives **IVc,IVf,IVi** more potent than aliphatic substation **IVa,IVh** .The major exception proved to be nitro derivatives **IVb,IVg** which being inactive at the used dose, in addition introduction CH_3 moiety into oxime derivative of pyrimidine ring abolish the anti-inflammatory activity of **Va-Vd**

P-chloro phenyl derivative **IVi** was more potent anti-inflammatory compound followed by methoxy phenyl derivatives **IVc,IVf**. While the phenyl, naphtlhyl derivatives **IVd,IVe** respectively, was found to be less active than their analogue chloro derivative **IVi**. This result revealed the importance of substituted phenyl moiety for the anti-inflammatory activity of the tested compounds. Furthermore, the order of activity chloro>methoxy>phenyl (naphthyl) derivative.

3.2.2. Analgesic activity: -

The analgesic activity of the synthesized compounds was assessed by the acetic acid-induced writhing method. According to structure-activity relationship (SAR) studies. The *p*-chloro phenyl derivative of the benzimidazole system **IVi** was shown very potent analgesic activity (77.04%) in comparison with Diclofenac sodium (85.24%), Replacement chloro moeity in **IVi** with methoxy moeity in benzimidazole derivative **IVc** and imidazopyridine derivative **IVf** lead to slightly decrease in analgesic activity, but **IVc,IVf** still showed potent analgesic activity (68.84%. 75.04%) respectively. *P*-Nitro phenyl derivative **IVb,IVg** and methoxy imino derivatives **Va-Vd** were showed week to non analgesic activity. That revealed the importance of substituted phenyl and the hydroxy-imino moieties of the imidazole system for the analgesic activity. The results are summarized in (Table 2, Fig.2)

3.2.3. Statistical analysis

Al values were expressed as Mean ± S.E.M. Statistical significant was determined using the student't-test.Values with P> 0.01

3.2.3. Anti-microbial activity: -

Compounds **IVc,IVd,IVe,IVf,IVh,IVi** and **Va,b,d,e** have been evaluated for their anti-microbial activity. The results of inhibition zone diameter (mm/mg) represent in (Table 3) Minimum Inhibitory Concentration (MIC) was also determined for the compound **IVh** that showed a good anti-microbial profile in the preliminary screening. It was interesting to note that compound **IVh**, which has ethyl moiety substituted with pyramidine ring of imidazole system was found to be the most potent as antibacterial agent with MIC (0.078µg/ ml) against S. aureus (a gram –

positive) (0.039µg/ml) against B. cereus (a gram –positive) and E. coli, S. typhimurium (a gram –negative) in comparison with Cefperazone which showed MIC (25µg/ml). In addition, **IVh**, exhibited anti -fungal activity with MIC (0.078µg/ ml) against C. albicans and (0.039µg/ml) against A. flavus. The reference antifungal drug Fluconazole showed MIC (12.5µg/ml). This activity may be attributed to presence pharmacologically active thio substituent attached to pyrimidine ring of benzimidazole system

Compounds **IVc,IVd** showed moderete antibacterial screening, while **IVe** exhibited antibacterial activity against a gram -positive bacteria only. The remaining compounds showed poor anti-microbial activity

3.3. Molecular docking result: -

The aim of the work was to study the crystal structure of COX-2 to rationalize the obtained biological data and explain the possible interactions that might take place between the tested derivatives and COX-2 enzyme in comparing to indomethacin (crystallized ligand) in order to obtain anti-inflammatory effect.

Molecular docking simulations were performed using FlexX. Leadit.2.1.2, while the tested compounds were visualized inside the pocket to view their fitting and closure to main residues by MOE 2010.10

FlexX. Leadit 2.1.2 allows the flexible docking of ligands into the site of action. It has the ability to use all the rotatable bonds of the ligands to give a number of conformations from which the best mode could be achieved. In the analysis of docking results we tried to find a correlation between the biological results and docking studies.

First, indomethacin interacted with Tyr 385,phe 381,Val 523,and Leu 532 by H-Arene interaction, while its –COOH group formed two H-bond with NH2 of Arg 120 in distance 2.46 A° and 2.82 A° (Fig 3).

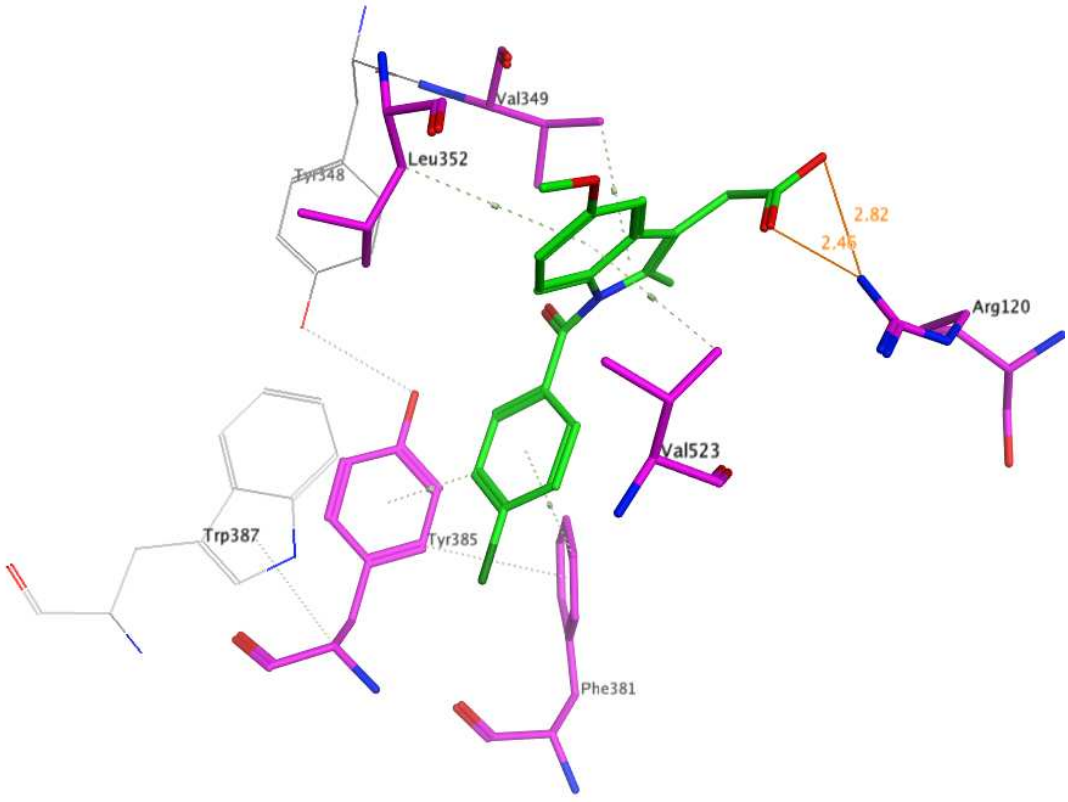


Fig. 3 showing Indomethacin (green color) interaction with amino acid residues of COX-2, Val523, Val349, phe381, Tyr385, Leu352 and Arg120 (purple color). Arg120 formed two H-bond with –CO group and –OH group of indomethacin carboxylic group in distance 2.46A°, 2.82A° respectively

It was found that, **IVi**, the best anti-inflammatory agent, formed four H-bonds, the imidazole ring and pyrimidine formed two H-bond with Val523, while *p*-chlorophenyl formed one H-bonds with Trp 387, and Phenyl ring

interacted with NH2 of Arg120 residue (Fig. 4-6). The *P*- chloro phenyl moiety of **IVi** and indomethacin embedded in the same hydrophobic region of pocket surface and interacted almost with the same amino acid residues (Fig.7).

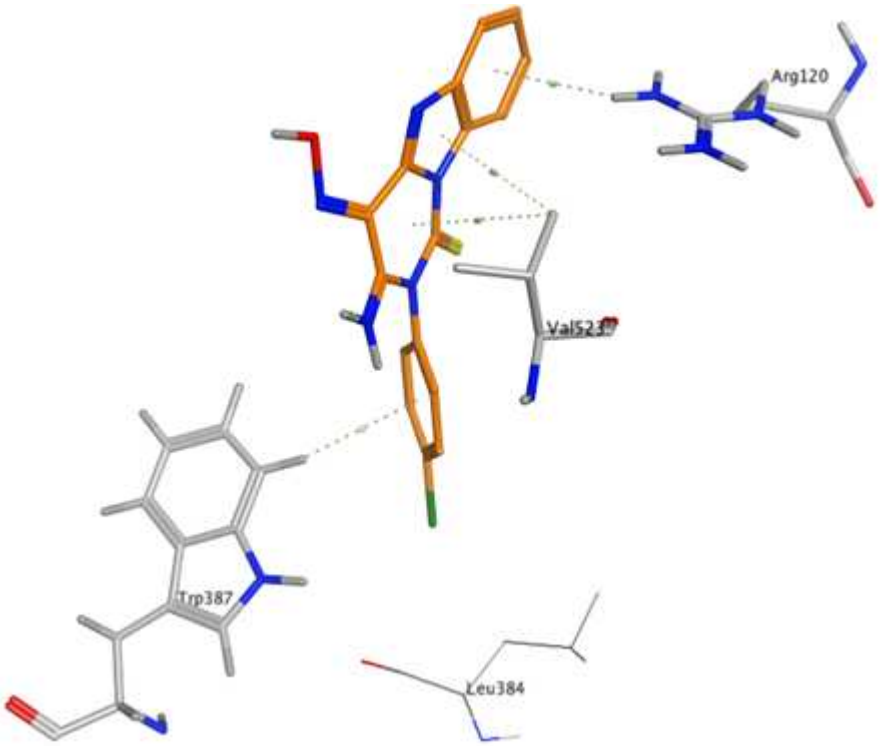


Fig. 4 Interaction of compound IVi (orange color) with Arg120, Val523, Trp387 in gray color

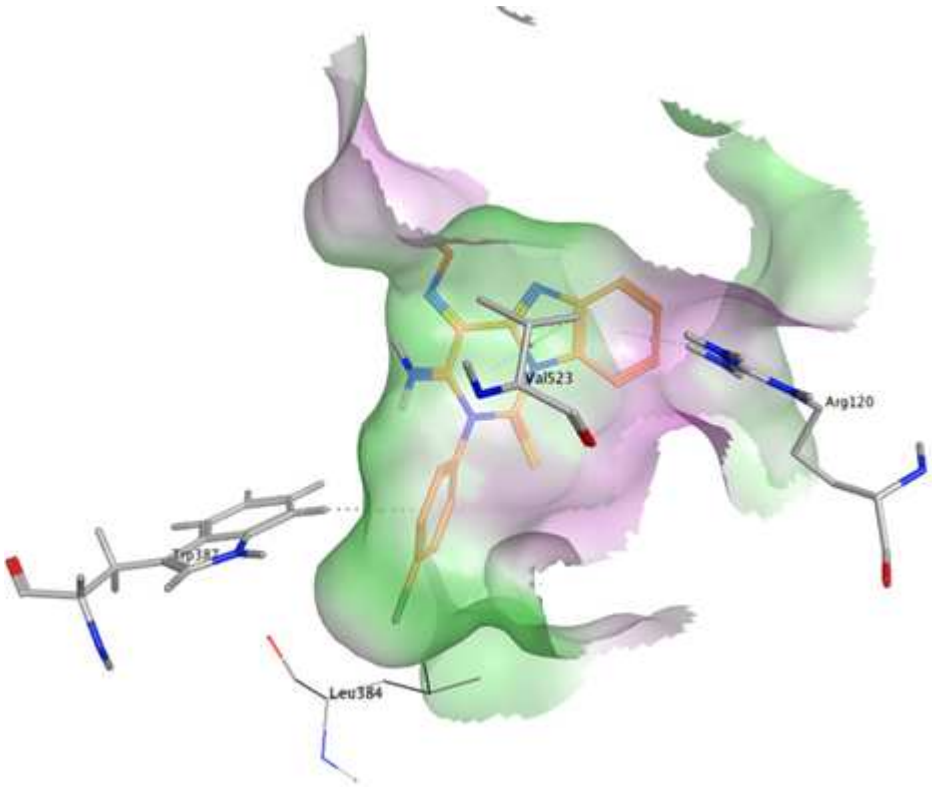


Fig. 5 Compound IVi (orange) deeply embedded into COX-2 binding domain, forming H-bonds with different residues (black dash line). Molecular surface of COX-2 colored as hydrophobic (green), hydrophilic (purple) and neutral (white).

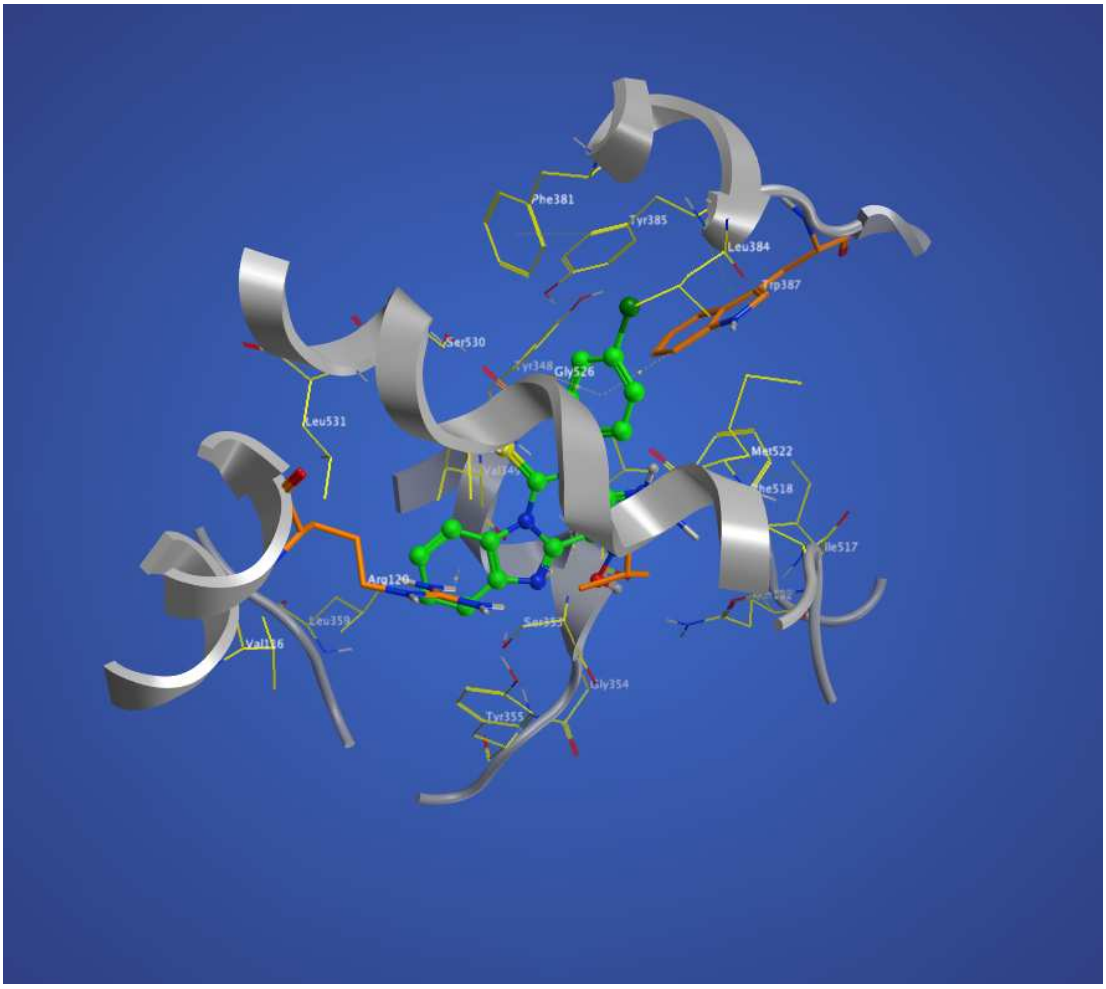


Fig. 6 Illustrates compound IVi (green) and its fitting in the protein pocket site, the protein is represent as cartoon and colored as gray, while the amino acid residues which interact with IVi colored as orange

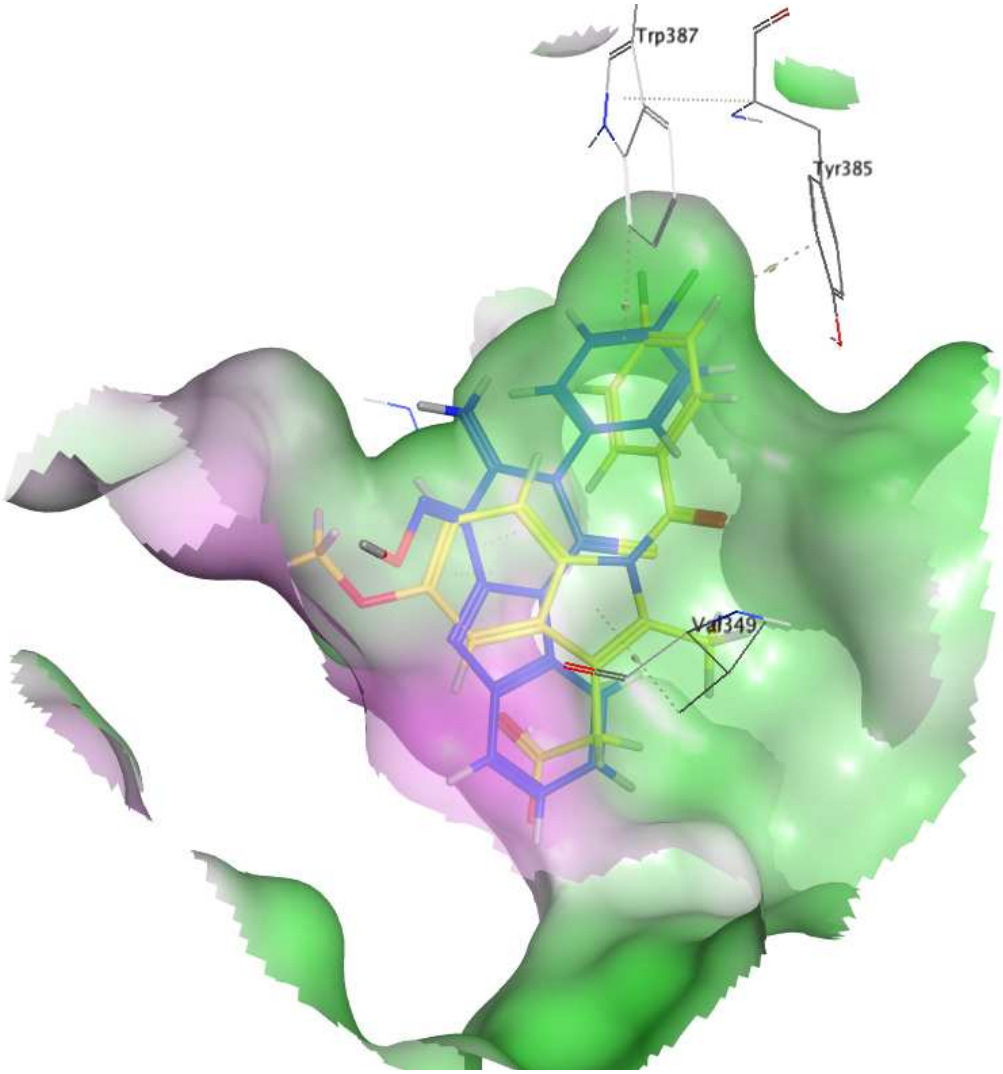


Fig. 7 Compound IVi (blue) superimposed into crystallized ligand, Indomethacin (yellow).In particular, the *p*-chloro phenyl of IVi and indomethacin embedded in the same hydrophobic surface of the pocket site
The two analogues IVc,IVf interacted with Arg120,Val 349, Val523,Trp387 but IVf. Its O-CH3 formed additional H-bond with -CO of Leu 352 in a distance (2.02 A°), (Fig. 8-11)

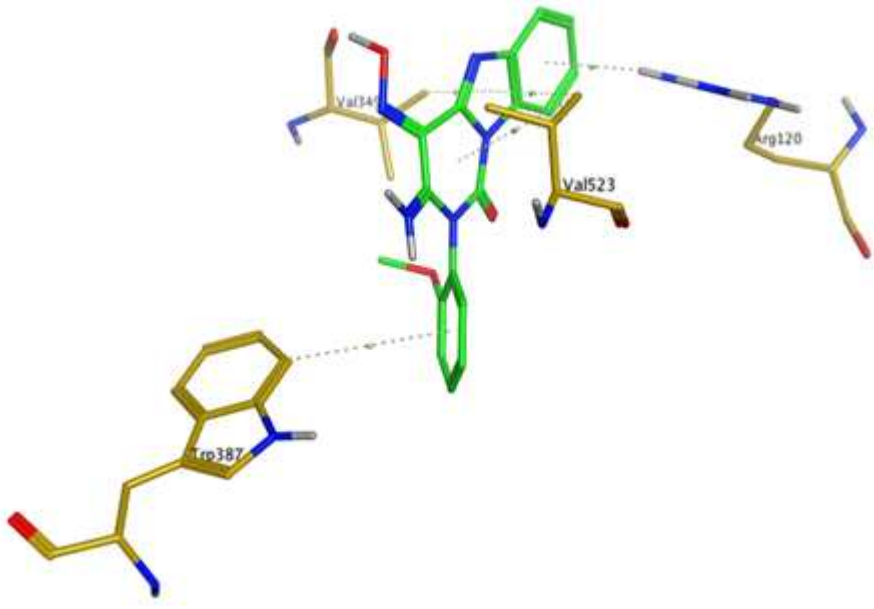


Fig. 8 Tthe possible H-bonds formation between compound IVc (green), and Arg120, Val 523,Val 349 and Trp 387(yellow).

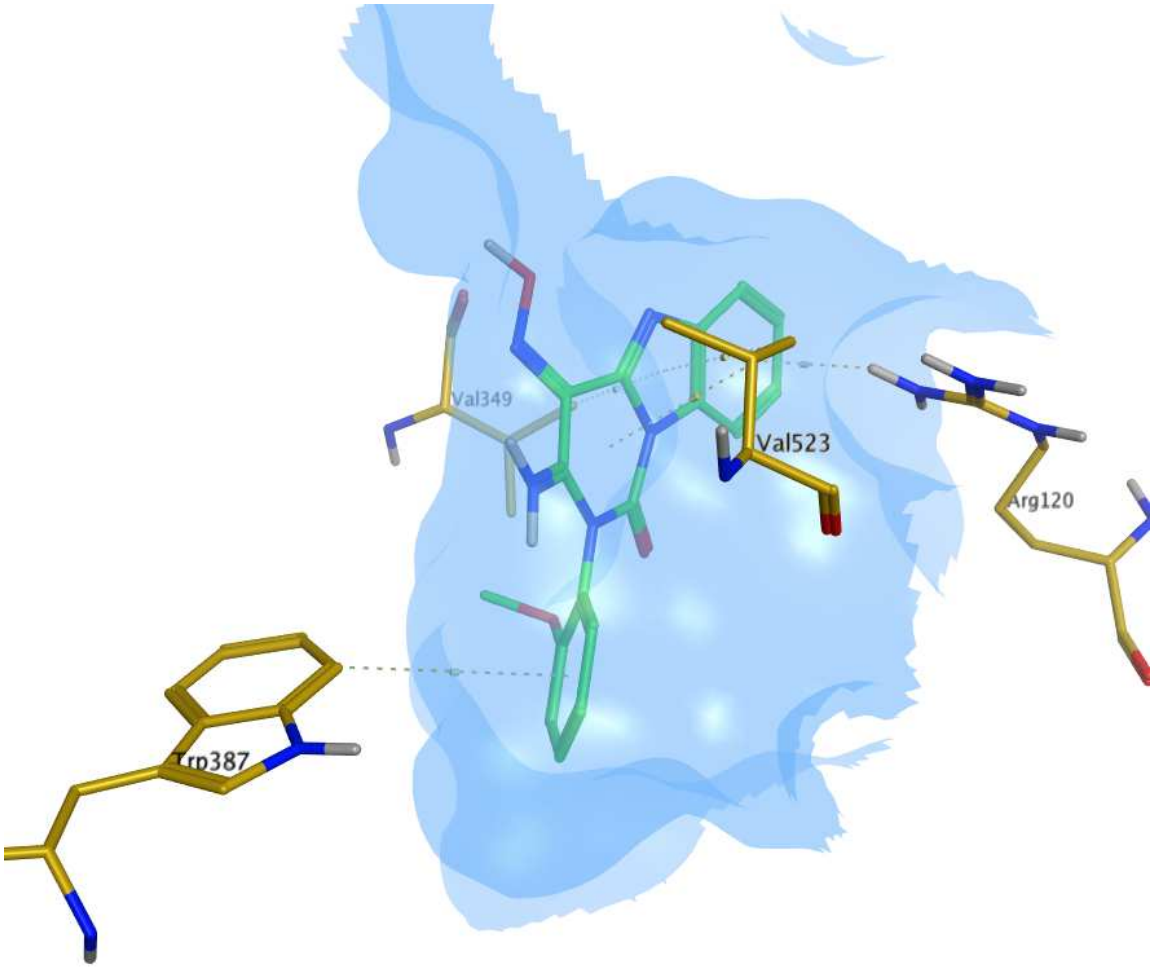


Fig. 9 Docking of compound IVc (green) in the binding site of the Cox-2 enzyme, the docked ligand exhibited H-bonds with almost same amino acids involved with indomethacin.

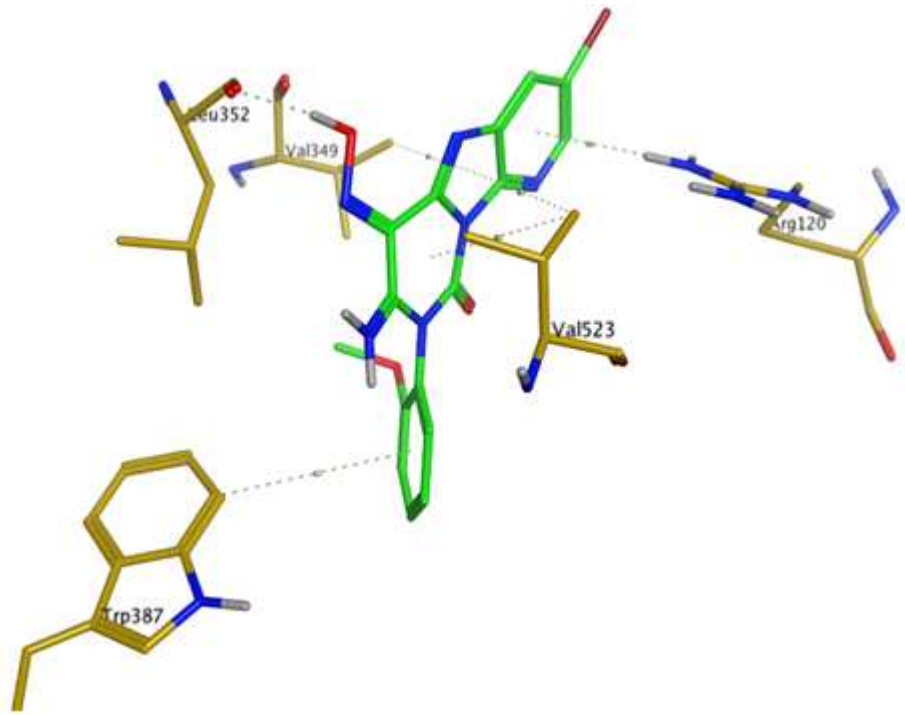


Fig. 10 H-bonds formation between IVf (green) and COX-2 pocket residues.

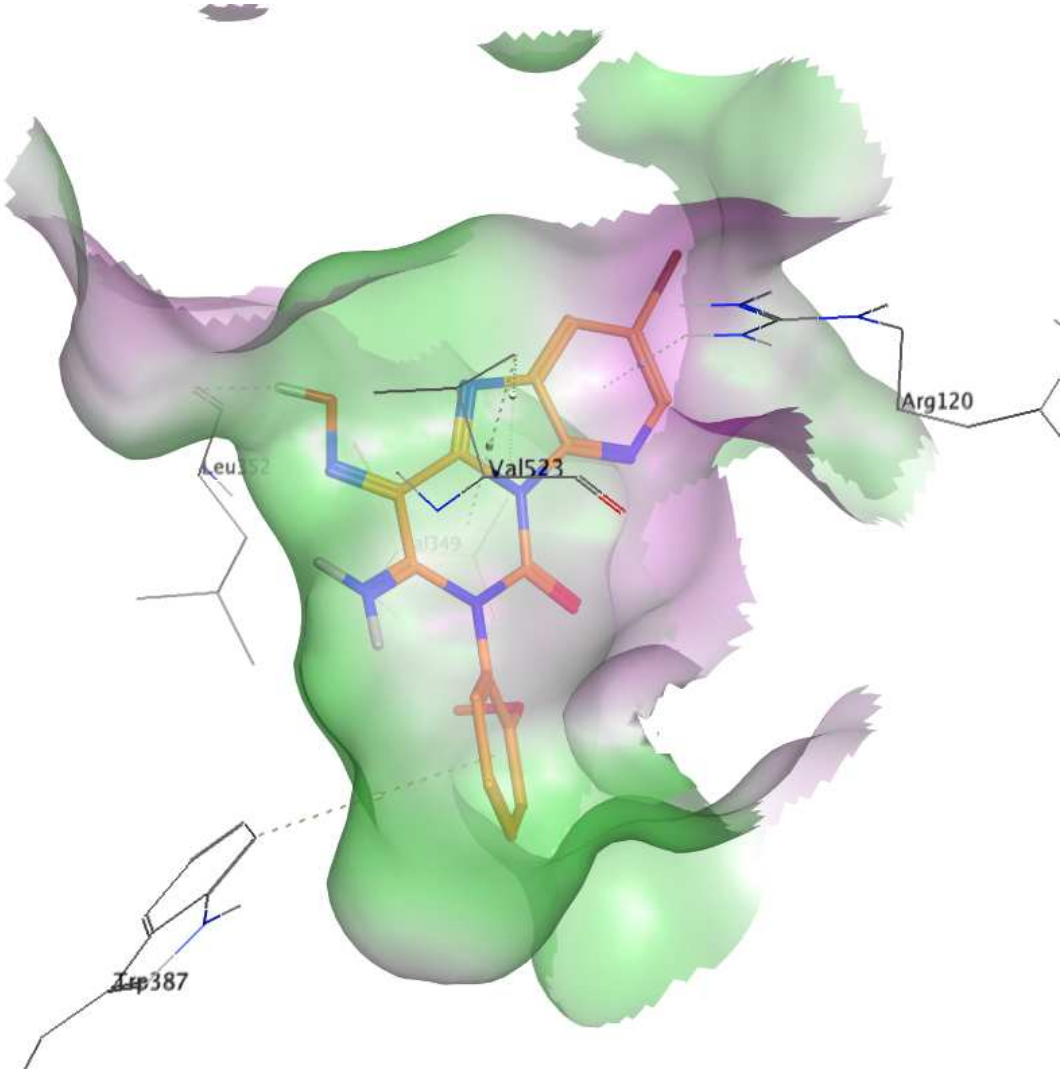


Fig. 11. Illustrates compound IVf (orange) and how it fits into the pocket site of COX-2. The molecular surface of the pocket site colored as hydrophobic (green), hydrophilic (purple), and neutral (white).

Two analogues **IVc,IVf** superimposed into COX-2 pocket site, showed nearly the same interaction . In particular *p*-methoxy phenyl of the two compounds was found in the same position of the hydrophobic molecular surface of COX-2. (Fig. 12)

Replacement Aromatic substitution of pyrimiine moiety by aliphatic substitution as **IVa,IVh** ,lead to bad fitting in the pocket site while the thio derivative **IVh** exhibited interaction with Val349,Val 523 and the phenyl group form H-arene bond with Leu352(not shown),this may be due to replacement Oxygen atom by Sulpher atom .Nitro derivatives **IVb, IVg** showed bad fitting, and **IVg** was out of the pocket, which correlate with the biological result

Methyl derivatives **Va,Vb,Vd,Ve** either out of the pocket site, or number of interaction with amino acid residues decreased or abolished completely in comparison to non-methyl derivatives

Its clear from molecular docking result there is good correlation with the biological result, and the molecular docking result, along with the biological assay data, suggesting that compounds **IVi** promising inhibitor of COX-2 enzyme

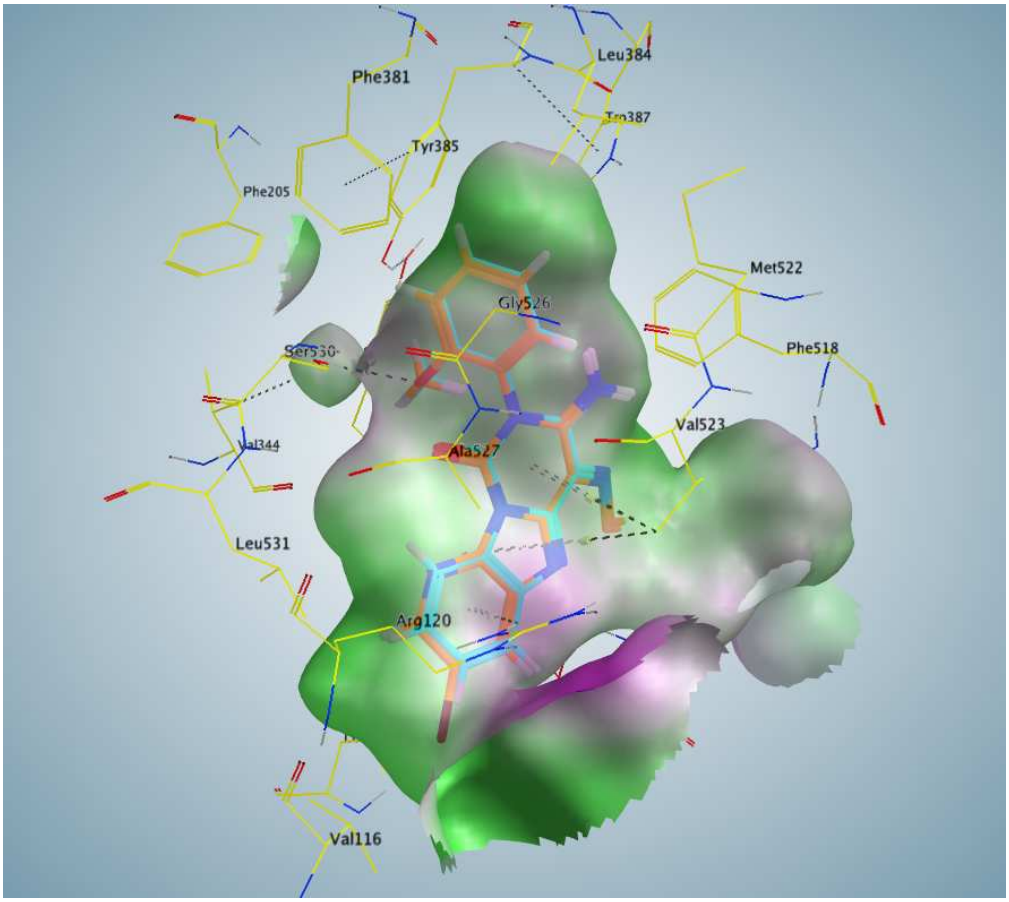


Fig. 12 Represents how the two analogues IVc (blue), IVf (orange) superimposed into COX-2 pocket site, exhibited almost the same H-bonds (in black dash line)

CONCLUSION

A new series of pyrimido [1,6-a] benzimidazole and pyrimido-imidazo [4,5-b] pyridine derivatives were synthesized with the objective of developing a new anti-inflammatory-antimicrobial agent with analgesic activity. Compounds **IVc,IVf** and **IVi** were found to be potent anti-inflammatory and analgesic agents with percent oedema inhibition (33.92%,33.92% and 37.63%) respectively. And protection percent as analgesic agents (68.84,75.04% and 77.04%) respectively. In particular compound **IVi** showed the most potent anti-inflammatory and analgesic activity. Moreover docking studies of compounds that have highest anti-inflammatory activity showed that compound **IVi** displayed stronger binding interactions with the active site of the human COX-2 enzyme. However, these derivatives did not show reasonable antimicrobial activity against all of the tested strains. Compound **IVh** was found to be the most active anti-microbial agent with MIC less than reference drugs in the present study

Therefore, as compound **IVh** exhibited moderate anti-inflammatory and analgesic activities. In addition potent anti-microbial activity, it can be concluded that compound **IVh** would constitute useful model for further investigation in the development of a new class of anti-inflammatory-antibacterial agents with analgesic activity.

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REFERENCES

[1] R. M. Garavito, *Nat. Struct. Biol*. **1996,** 3, 897.
[2] J. R. Vane, *Nature*. **1971**, 231, 232.
[3] C. Bombardier, L. laine, A. Reicin, D. Shapiro, R. Burgos-Vargas, *Eng. J. Med*. **2000**, 343,1520.
[4] F. E. Silverstien, G. Faich, J. L. Goldstien, L. S. Simon, T. Pincus, A. Whelton, R. Makuch, G. Eisen, *J. Am. Med. Assoc*. **2000**, 284,1247.

[5] N. Terioglu, A. Gursoy, *J. Med. Chem*. **2003**, 38, 781
[6] A. K. Gadad, M. N. Noolvi, R. K. Karpoormath, *Bioorg. Med. Chem. **2004,** 12, 5651*.
[7] A. Andreani, M. Granaiola, A. Leoni, A. Locatelli, R. Morigi, G. Linaz, C. Bergamini, G. Farruggia, *J. Med. Chem*. **2005,** 48, 3085.
[8] A. Andreani, A. Leoni, A. Locatelli, R. Morigi, M. Rambaldi, M. Recanatini,V. Garaliene, *Bioorg. Med. Chem*. **2000**, 8, 2359.
[9] D. Zhang, Z. Wang, W. Xu, F. Sun, L. Tang,Wang, J. *Eur. J. Med. Chem.* **2009**, 44, 2202.
[10] T. H. Al-Tela, R. A. Al-Qawasmeh , *Eur. J. Med. Chem*. **2010**, 45, 2048.
[11] K. C. S. Achar , K. M. Hosamani, H. R. Seetharamareddy, *Eur. J. Med. Chem*. **2010**, 45, 2048.
[12] M. Mader, A. de Dios, C. Shih, R. Bonjouklian, T. Li, *Bioorg. Med. Chem. Lett*. **2008**, 18, 179.
[13] S. Demirayak, I. Kayagil, L. Yurttas, *Eur. J. Med. Chem*. **2011**, 46, 411.
[14] T. D.; Penning, D. Zhu, V. B. Gandhi, J. Gong, S.Thomas, W. Lubisch, R. Grandel, W. Wernet, C. H. Park, *Bioorg. Med. Chem.* **2008**, 16, 6965.
[15] A. Gellis,´ H. Kovacic, N. Boufatah, p Vanelle, *Eur. J. Med. Chem*. **2008**, 43, 1858.
[16] F. Hernández-Luis, A. Hernández-Campos. R. Castillo, G. Navarrete- Vázquez, O. Soria-Arteche, M. Hernández-Hernández, L. Yépez-Muli, *Eur. J. Med. Chem*. **2010**, 45, 3135.
[17] K. Yu, Y. Sin, R. L. Civiello, X. Alan Wang, K. D. Combrink, H. B. L. Belgin Gulgeze, J. J. Kim Wright, R. A. Dalterio, *Bioorg. Med. Chem. Lett*. **2007**, 17, 895.
[18] S. Lee, H. T. Kim, C. Park, D. Lee, H. Chang, S. Park, C. M. Cho, S. Ro, Y. Suh, *Bioorg. Med. Chem. Lett*. **2012**, 22, 4221.
[19] X. J. Wang. M. Y. Xi, J. H.; Fu, F. R. Zhang, G. F. Cheng, d. L. Yin, Q. D. You,*Chin. Chem. Lett.* **2012**, 23, 707.
[20]. O. Kim, Y. Jeong, H. Lee, S. Hong, S. Hong, *J. Med. Chem*. **2011**, 54, 2455.
[21] A. Scribner, R. Dennis, S. Lee, G. Ouvry, D. Perrey, M. Fisher, M. Wyvrat, p. Leavitt, P. Liberator, *Eur. J. Med. Chem*. **2008**, 43, 1123.
[22] T. Biftu, D. Feng, M. Fisher, G. Liang, X. Qian, A. Scribner, R. Dennis, S. Lee, P. A. Liberator, *Bioorg. Med. Chem. Lett*. **2006**, 16, 2479.
[23] H. S. Patel, j. A. Linn, D. H. Drewry, D. A. Hillesheim, W. J. Zuercher, W. J. Hoekstra, *Tet. Lett*. **2003**, 44, 4077.
[24] K. S. Gudmundsson, S. D. Boggs, J. g. Catalano, A. Svolto, A. Spaltenstein, M.Thomson, P. Wheelan, S. Jenkinso, *Bioorg. Med. Chem. Lett*. **2009**, 19, 6399.
[25] http/www/BiSolveit GmbH-Leadit
[26] http/www/chemical computing group
[27] A. M. Nawwar, L. M. Chabaka.*Phosphorus, Sulfur and Silicon*, **1991**, 57,65
[28] S. I. Alqasoumi, A. M. Al-Taweel A. M. Alafeefy, E. Noaman, M. M. Ghorab, *Eur. J. Med. Chem*. **2010**, 45, 738.
[29] C. A. Winter, E. A.; Risley, G. W. Nuss, Proc. *Soc. Exp*. **1962**, 111, 550
[30] S. H. Gillespie, medical microbiology-Illustrated. Butterworth Heinemann LTD., united Kindgom, **1994**, 234.