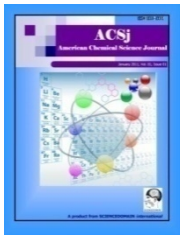

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Synthesis, Antiviral and Kinesin Eg5 Activities of New Indomethacin Analogues

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Authors' contributions

This work was carried out in collaboration between both authors. Author NAl-M designed the study, wrote the protocol, managed the analyses of the study, managed the literature searches and wrote the first draft of the manuscript. Author DSA synthesized the new compounds. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: Synthesis, characterization, anti-HIV, anti-HCV and kinesin activities of new indomethacin analogues have been carried out.
Methodology: Arylated derivatives of indomethacin via the Suzuki-Miyaura cross-coupling reaction using palladium acetate/triphenylphophineor palladium based *N*-heterocyclic carbene (Pd-NHC) complexe as catalysts were synthesized and characterized by the ^{1}H and ^{13}C and 2D NMR study. Analogously, indomethacin analogues bearing thioureido and amide moieties of various L-amino acid esters were prepared *via* Kabbani and coupling reactions, respectively.
Results: All the new analogues were evaluated *In vitro* for their antiviral activity against the replication of HIV-1 and HIV-2 in MT-4 cells using MTT assay. Compounds 28, 31 and 32 were evaluated *In vitro* for their inhibitory activity against hepatitis virus C (HCV) in the Huh 5-2 replicon system (type 1b, Con1 strain). Additionally, some analogues were screened for their inhibitory activity against the ATPase enzyme and the motor-protein Kinesin Eg5.In conclusion, Compounds 31 and 39 showed anti-HIV activity with IC_{50} values of >1.81 and > 3.21μM (CC_{50} of 3.31 and 28.89μM), resulting in selectivity indexs (SI) of 6 and 9, respectively.
Conclusion: Compounds 31 and 39 displayed better anti-HIV activity than the other derivatives (SI = 6 and 9, respectively). Compound 27 showed ATPase inhibition value of 48% at 100 μM concentration.

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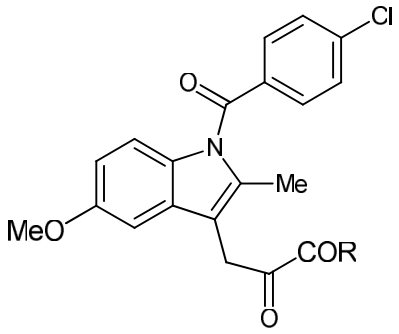
Keywords: Antiviral activity; anti-kinesin Eg5 inhibitors; indomethacin; suzuki-miyaura cross-coupling reaction; thioureido derivatives.

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are considered to be the most important therapeutic agents for the treatment of pain and inflammation related to a large variety of pathologies like rheumatoid arthritis and osteoarthritis. Such drugs are indomethacin, 1[1,2] etodolac [3] and tenidap [4]. Indomethacin, as one of NSAIDs, blocks the enzymes that make prostaglandins (cyclooxygenase (COX-1 and 2) and thereby reduces the levels of prostaglandin (PG), leukotriene and thromboxane precursors [5-8]. As a result, fever, pain and inflammatory rheumatoid diseases are reduced. Marnett et al. [9-12] reported that substitution of the 2′-methyl group of indomethacin with trifluoromethyl produces CF_3-indomethacin, a tight-binding inhibitor with kinetic properties, showed unexpected COX-2 selectivity in comparison to indomethacin itself (IC_{50} mCOX-2 = 267 nM, IC_{50} COX-1 > 100 μM). Furthermore, COX inhibitors, indomethacin and its structural analogue sulindac exhibit cell growth inhibition and apoptosis inducing activities in various cancer cell lines *via* COX independent mechanisms. [13] On the other hand, it has been found that removal of the methyl group from 1 would lead drastically in reduction of the inhibitory potency against both COX enzymes [14].

Recently, Kalgutkar et al. [15,16] have reported that derivatization of indomethacin carboxylic acid group to ester or amide analogues produces compounds would lead to highly selective and potent COX-2 inhibitors, while other analogues having *N*-difluoromethyl-1,2-dihydropyrid-2-one moiety [17] reported as cyclooxygenase and lipoxygenase inhibitors. Zorc et al. [18] have examined some NSAID hydroxamic acids against malignant tumor cell lines and normal human fibroblasts and found indomethacin analogue 2 exhibited a moderate inhibition against breast carcinoma, murine leukemia and human T lymphocytes (mean IC_{50} = 4.2-28 μM). In addition, alkyl-tin carboxylate derived from indomethacin exhibited remarkable *In vitro* cytotoxicity [19], while some new indomethacin analogues have been reported as potent antitumor agents [20,21], with remarkable cytotoxic activity(e.g. 3) [22]. Unexpectedly, Santoro et al. [23] have found that indomethacin has a potent antiviral activity against the coronaviruses SARS-CoV. In 2005, Richardson et al. [24]have reported that indomethacin is a potential therapeutic target in respiratory syncytial virus **(**RSV) therapy, meanwhile Mukherjee and Simpson [25] have indicated the effect of indomethacin *In vitro* on the vesicular stomatitis virus. Furthermore, Bourinbaiar and Lee-Huang [26] have studied the anti-HIV activity of anti-inflammatory drugs, dexamethasone and indomethacin, by MAP30, the antiviral agent from bitter melon.

In continuation of our ongoing work on the synthesis of new anti-HIV agents and our recent antiviral data on new indomethacin derivatives [27], we report here the synthesis of new indomethacin analogues with evaluation of their anti-HIV, anti-HCV and anti-kinesin Eg5 activity.



1. R = OH (indomethacin)
2. R = $(CH_2)_2CO_2H$
3. R = NHOH

2. EXPERIMENTAL

2.1 Materials and Methods

Melting points are uncorrected and were measured on a Büchi melting point apparatus B-545 (BÜCHI Labortechnik AG, Switzerland). NMR data were obtained 400 and 600 MHz (^1H) and 150.91 MHz (^{13}C) spectrometers (Avance III, Bruker, Germany) with TMS as internal standard and on δscale in ppm. All nmr spectra were measured in dimethyl sulfoxide d_6. Heteronuclear assignments were verified by ^1H-^{13}C COSY, or HMQC experiments. Microanalytical data were obtained with a Vario, Elemental apparatus (Shimadzu, Japan). Mass spectra were recorded on 70 eV EI and FAB MAT 8200 spectrometers (Finnigana MAT, USA). Silica gel (0.040-0.063 mm) used for column chromatography and analytical silica gel TLC plates 60 F254 were purchased from Merck.

2.2 Synthesis of the Compounds

2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-indol-3-yl)acetyl chloride (4)
This compound was prepared according to the reported procedure [15]:

2.2.1 General procedure of preparation of indomethacin having triaryl groups *via* Suzuki-Miyaura cross-coupling reaction [17-27]

Method A. A mixture of indomethacin 1 (100 mg, 0.28 mmol) and arylboronic acids 5-16 (0.29 mmol) in *n*-propanol (15 mL) was stirred for 15 min, and to this mixture was added $Pd(OAc)_2$ (19 mg, 0.085 mmol), triphenylphosphene (66mg, 0.25 mmol) and 2m aq. solution of Na_2CO_3 (3.5 mL). The reaction mixture was refluxed for 4-6 h and completion of reaction was monitored by TLC. After cooling, water was added (7 mL), followed by stirring for 5 min and the mixture was partitioned with ethyl acetate (3×10 mL). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated to dryness. The crude product was purified on a column of SiO_2 (5 g) using: hexane-EtOAc = 3:2 or, in gradient, MeOH (0-10%) and $CHCl_3$ as eluents to give the desired product.

Method B. A mixture of indomethacin 1 (358 mg, 1.0 mmol), arylboronic acids 5-7 (1.2 mmol), Pd-NHC catalyst (1 mol %, 0.0096 g), K_2CO_3 (276 mg, 2.0 mmol), and a mixture of

acetone/water (1:1) (10 mL) was stirred at 40 °C for 3 h The reaction mixture was then diluted with water (20 mL) and extracted three times with dichloromethane (3×10 mL). The combined organic layer was washed with brine (20 mL) and dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness. The crude products was purified on a SiO_2 as in method A to give 17-19. The physical data were identical for those compounds prepared previously in method A.

2-(1-(2'-Fluoro-[1,1'-biphenyl]4-carbonyl)-5-methoxy-2-methyl-indol-3-yl)aceticacid (17):

From 2-fluorophenylboronic acid 5 (39 mg). Yield: 123 mg (88%), as a yellow powder, mp 198-200 ^{o}C.^{1}H NMR (DMSO-d_6): δ 10.42 (s, 1H, CO_2H), 7.88 (dd, 2H, J = 7.0, 1.8 Hz, 2'-H_{arom} + 6'-H_{arom}), 7.74 (m, 1H, 6''-H_{arom}), 7.64-7.49 (m, 3H, 3'-H_{arom} + 5'-H_{arom} + 4''-H_{arom}) , 4.32 (m, 2H, 3''-H_{arom} + 5''-H_{arom}), 7.05 (d, 1H, J= 8.1 Hz, 7-H_{indole}), 6.96 (d, 1H, J = 2.1 Hz, 4-H_{indole}), 6.54 (dd, 1H, J = 8.1, 2.4 Hz, 6-H_{indole}), 3.71 (s, 3H, OMe), 3.48 (s, 2H, CH_2), 2.28 (s, 3H, Me). ^{13}C NMR (DMSO-d_6): δ 176.0 (CO_2H), 168.9 (C=O), 157.5 (*C*-OMe + C-F), 142.6 (C4'$_{arom}$), 138.9 (C3a$_{indole}$), 133.5 (C2$_{indol}$), 132.4, 131.4, 130.8, 129.5, 128.6, 127.0 (C$_{arom}$), 124.9 (d, $J_{C,F}$ = 120 Hz, C1''$_{arom}$), 117.4 (d, $J_{C,F}$ = 240 Hz, (C3''$_{arom}$), 110.2 (C7$_{indol}$), 108.7 (C3$_{indol}$), 108.3 (C6$_{indol}$), 100.8 (C4$_{indol}$), 55.3 (OMe), 30.7 (CH_2), 11.6 (Me). Anal. calcd for $C_{25}H_{20}FNO_4$ (417.43): C, 71.93; H, 4.83; N, 3.36. Found: C, 71.71; H, 4.73; N, 3.09.

2-(1-(3'-Fluoro-[1,1'-biphenyl]4-carbonyl)-5-methoxy-2-methyl-indol-3-yl)aceticacid (18):

From 3-fluorophenylboronic acid 6 (39 mg). Yield: 96 mg (82%), as a yellow powder, mp 200-202 ^{o}C.^{1}H NMR (DMSO-d_6): δ 10.41 (s, 1H, CO_2H), 7.89 (dd, 2H, J = 6.9, 1.9 Hz, 2'-H_{arom} + 6'-H_{arom}), 7.66-7.55 (m, 4H, 3'-H_{arom} + 5'-H_{arom} + 2''-H_{arom} + 4''-H_{arom})7.32 (dd, 2H, J = 8.3, 2.3 Hz, 5''-H + 6''-H_{arom}), 7.06 (d, 1H, J= 8.6 Hz, 7-H_{indole}), 6.96 (d, 1H, J = 2.4 Hz, 4-H_{indole}), 6.55 (dd, 1H, J = 8.6, 2.4 Hz, 6-H_{indole}), 3.71 (s, 3H, OMe); 3.48 (s, 2H, CH_2), 2.28 (s, 3H, Me). ^{13}C NMR (DMSO-d_6): δ 176.0 (CO_2H), 168.8 (C=O), 161.8 (d, $J_{C,F}$= 257 Hz, C-F), 161.2 (*C*-OMe), 146.5 (C-4'$_{arom}$ + C1''$_{arom}$), 139.0 (C3a$_{indol}$), 133.4 (C2$_{indol}$), 132.1, 132.0, 131.4, 130.8, 130.1, 129.4, 128.7, 126.9 (C$_{arom}$), 123.2 (d, $J_{C2'',F}$ = 120 Hz, C2''$_{arom}$), 116.4 (d, $J_{C4'',F}$= 120 Hz, C4''$_{arom}$), 114.8 (C3$_{indol}$),110.2 (C7$_{indol}$), 108.6 (C6$_{indol}$), 100.8 (C4$_{indol}$), 55.2 (OMe), 30.8 (CH_2), 11.1 (Me). Anal. calcd for $C_{25}H_{20}FNO_4$ (417.43): C, 71.93; H, 4.83; N 3.36. Found: C, 71.73; H, 4.69; N, 3.18.

2-(1-(4'-Fluoro-[1,1'-biphenyl]4-carbonyl)-5-methoxy-2-methyl-indol-3-yl)aceticacid (19):

From 4-fluorophenylboronic acid 7 (39 mg). Yield: 120 mg (86%), as a yellow product, mp250-252°C. ^{1}H NMR (DMSO-d_6): δ 10.45 (s, 1H, CO_2H), 7.90 (dd, J = 8.5, 2.2 Hz, 2'-H_{arom} + 6'-H_{arom}), 7.66-7.56 (m, 2H, 2''-H_{arom} + 6''-H_{arom}), 7.34-7.31 (m, 4H, 3'-H_{arom} + 5'-H_{arom} + 3''-H_{arom} + 5''-H_{arom}), 7.06 (d, 1H, J = 8.4 Hz, 7-H_{indol}), 7.05 (d, 1H, J = 2.2 Hz, 4-H_{indol}), 6.59 (dd,1H, J = 8.4 Hz, 6-H_{indol}), 3.70 (s, 3H, OMe), 3.25 (s, 2H, CH_2), 2.27 (s, 3H, Me). ^{13}C NMR (DMSO-d_6): δ 175.9 (CO_2H), 168.9 (C=O), 161.5 (d, $J_{C4'',F}$= 252 Hz, C4''$_{arom}$-F), 160.8 (*C*-OMe), 145.8 (C4'$_{arom}$), 138.9 (C3a$_{indol}$), 133.4 (C1''$_{arom-F}$), 132.4 (C2$_{indol}$), 131.4, 130.8, 129.4, 128.6, 127.0 (C$_{arom}$), 118.1 (2d, J = 121 Hz, C3''$_{arom}$ + C5''$_{arom}$), 116.0 (C3$_{indol}$), 114,1 (C7$_{indol}$), 110.2 (C6$_{indol}$), 100.0 (C4$_{indol}$), 55.2 (OMe), 33.8 (CH_2), 11.6 (Me). Anal. calcd for $C_{25}H_{20}FNO_4$ (417.43): C, 71.93; H, 4.83; N, 3.36. Found: C, 71.69; H, 4.77; N, 3.12.

2-(1-(3',4'-Difluoro-[1,1'-biphenyl]4-carbonyl)-5-methoxy-2-methyl-indol-3-yl)aceticacid (20):

From 3,4-difluorophenylboronic acid 8 (44 mg). Yield: 112 mg (78%), as a yellow powder, mp 190-192°C. ^{1}H NMR (DMSO-d_6): δ 10.42 (s, 1H, CO_2H), 7.89 (dd, 2H, J = 8.6, 2.2 Hz, 3'-

Harom + 6'-Harom), 7.64-7.53 (m, 1H, 6''-Harom-F), 7.32 (d, *J* = 8.6 Hz, 2H,2'-H_{arom} + 6'-H_{arom}), 7.61 (m, 1H, 6''-H_{arom}-F), 7.55 (dd, 2H, *J* = 8.6, 2.2 Hz, 2'-Harom + 5'-Harom), 7.31 (m, 1H, 5''-H_{arom}),7.06 (d, 1H, *J* = 8.0 Hz, 7-Hindol),6.94 (d, 1H, *J* = 2.1 Hz, 4-Hindol), 6.66 (m, 1H, 6''-H_{arom}), 6.54 (dd, 1H, *J* = 8.0, 2.0 Hz 6-H_{indol}), 3.70 (s, 3H, OMe), 3.47 (s, 2H, CH_2), 2.27 (s, 3H, Me). ^{13}C NMR (DMSO-d_6): *δ* 176.1 (CO_2H), 168.9 (C=O), 154.1 (*C*-OMe), 152.4 (C4'$_{arom}$), 148.4 (d, $J_{C3'',F}$ = 256 Hz, C3''$_{arom}$), 140.8 (d, $J_{C4'',F}$ = 255 Hz, C4''$_{arom}$), 138.8 (C3aindol), 133.4 (C2indol), 132.3, 131.4, 130.1, 129.4, 128.6 (C_{arom}), 123.1 (d, $J_{C2'',F}$ = 122 Hz, C2''$_{arom}$), 121.5 (d, $J_{C5'',F}$ = 120 Hz, C5''$_{arom}$), 116.0 (C3indol), 115.4 (C7$_{indol}$), 110.2 (C6$_{indol}$), 100.9 (C4indol), 30.8 (CH_2), 55.2 (OMe). Anal. calcd for $C_{25}H_{19}F_2NO_4$ (435.42): C, 68.96; H, 4.40; N, 3.22. Found: C, 68.69; H, 4.32; N, 3.01.

2-(1-(3',4'-Dimethoxy-[1,1'-biphenyl]4-carbonyl)-5-methoxy-2-methyl-indol-3-yl)aceticacid (21):

From 3,4-dimethoxyphenylboronic acid 9 (51 mg). Yield: 138 mg (91%), as a brown powder, mp 210-212^{o}C.^{1}H NMR DMSO-d_6): *δ* 10.42 (s, 1H, CO_2H), 7.88 (dd, 2H, *J* = 8.5, 2.3 Hz, 2'-Harom + 6'-Harom), 7.62 (m, 1H, 2''-H_{arom}), 7.32 (dd, 2H, *J* = 8.5, 2.3 Hz, 3'-H_{arom} + 5'-H_{arom}), 7.09 (d, 1H, *J* = 8.2 Hz, 7-H_{indol}), 7.04 (d, 1H, *J* = 2.1 Hz, 4-H_{indol}), 6.94 (d, 1H, *J* = 8.0 Hz, 3''-H_{arom}), 6.55 (br s., 1H, 6''-H_{arom}), 6.53 (dd, 1H, *J* = 8.2, 2.1 Hz, 6-H_{indol}), 3.83, 3.77 (2xs, 6H, OMe), 3.42 (s, 2H, CH_2), 2.27 (s, 3H, Me). ^{13}C NMR (DMSO-d_6): *δ* 175.9 (CO_2H), 168.8 (C=O), 161.6 (*C5*-OMe), 152.4 (*C5''*-OMe), 148.9 (*C4''*-OMe), 147.9 (C4'$_{arom}$), 139.0 (C1''$_{arom}$), 133.3 (C(3a)$_{indol}$), 132.2 (C(2)$_{indol}$), 130.7, 130.1, 127.0 C_{arom}), 120.6 (C2''$_{arom}$), 114.3 (C3$_{indol}$ + C7$_{indol}$), 112.1 (C6''$_{arom}$), 110.1 (C6$_{indol}$), 108.5 (C3''$_{arom}$), 100.8 (C(4)$_{indol}$), 55.5, 55.2 (3xOMe), 30.8 (CH_2), 11.6 (Me). Anal. calcd for $C_{27}H_{25}NO_6$ (459.17): C, 70.58; H, 5.48; N, 3.05l. Found: C, 70.32; H, 5.40; N, 2.85.

2-(5-Methoxy-2-mehyl-1-(2'-(methylthio)-[1,1'-biphenyl]4-carbonyl)-indol-3-yl)aceticacid (22):

From 2-methylthiophenylboronic acid 10 (47 mg). Yield: 106 mg (72%), as a colourless powder, mp 238-240 ^{o}C.^{1}H NMR (DMSO-d_6): *δ* 10.42 (s, 1H, CO_2H), 8.11 (dd, 2H, *J* = 8.0, 2.1 Hz, 2'-Harom + 6'-Harom), 7.88 (dd, 2H, *J* = 8.0, 2.1 Hz, 3'-H_{arom} + 5'-Harom), 7.58 (m, 2H, 3''-H_{arom} + 6''-Harom), 7.31 (dd, 2H, 4''-H_{arom} + 5''-Harom), 7.05 (d, 1H, *J* = 8.4 Hz, 7-Hindol), 6.95 (d, 1H, *J* = 2.2 Hz, 4-Hindol), 6.53 (dd, 1H, *J* = 8.4, 2.1 Hz, 6-Hindol), 3.70 (s, 3H, OMe), 3.44 (s, 2H, CH_2), 2.50 (s, 3H, SMe), 2.27 (s, 3H, Me). ^{13}C NMR (DMSO-d_6): *δ* 176.0 (CO_2H), 168.8 (C=O), 160.8 (*C*-OMe), 142.5 (C4''arom), 138.9 (C-SMe), 133.4 (C3aindol + C2indol), 132.3, 131.5, 130.6, 129.5, 128.7, 127.0 (Carom), 114.1 (C3indol + C7indol), 108.7 (C6indol), 100.9 (C4indol), 55.2 (OMe), 34.2 (CH_2), 14.9 (SMe), 11.6 (Me). Anal. calcd for $C_{25}H_{23}NO_4S$ (445.53): C, 70.09; H, 5.20; N, 3.14. Found: C, 69.85; H, 5.11; N, 2.89.

2-(5-Methoxy-2-methyl-1-(4'-nitro-[1,1'-biphenyl]4-carbonyl)-indol-3-yl)acetic acid (23):

From 4-nitrophenylboronic acid 11 (47 mg). Yield: 120 mg (81%), as a brown powder, mp 198-200 ^{o}C.^{1}H NMR (DMSO-d_6): *δ* 10.42 (s, 1H, CO_2H), 8.34 (dd, 2H, *J* = 8.1, 2.0 Hz, 3''-$H_{arom\text{-}NO2}$ + 5''-$H_{arom\text{-}NO2}$), 7.89 (dd, 2H, *J* = 8.3, 2.3 Hz, 2'-Harom + 6'-Harom), 7.62-6.95 (m, 4H, 3'-Harom + 5'-Harom +2''-$H_{arom\text{-}NO2}$ + 6''-$H_{arom\text{-}NO2}$), 6.94 (d, 1H, *J* = 8.3 Hz, 7-H_{indol}), 6.89 (d, 1H, *J* = 2.2 Hz, 4-H_{indol}), 6.54 (dd, 1H, *J* = 2.2, 8.3 Hz, 6-H_{indol}), 3.70 (s, 3H, OMe), 3.44 (s, 2H, CH_2), 2.27 (s, 3H, Me). ^{13}C NMR (DMSO-d_6): *δ* 176.1 (CO_2H), 169.0 (C=O), 160.7 (*C*-OMe), 147.5 (C1''$_{arom\text{-}NO2}$), 146.8 (C-NO_2), 146.2 (C4'$_{arom}$), 135.9 (C3a$_{indol}$), 132.3 (C2$_{indol}$), 131.4, 130.8, 128.7, 127.3, 124.0 (C_{arom}), 114.0 (C3$_{indol}$ + C7$_{indol}$), 110.2 (C6$_{indol}$), 100.8 (C4$_{indol}$), 55.2 (OMe), 30.7 (CH2), 11.6 (Me). Anal. calcd for $C_{25}H_{20}N_2O_6$ (444.44): C, 67.56; H, 4.54; N, 6.30. Found: C, 67.32; H, 4.48; N, 6.02.

2-(1-(4'-(Ethoxycarbonyl)-[1,1'-biphenyl]4-carbonyl)-5-methoxy-2-methyl-indol-3-yl)aceticacid (24):

From 4-ethoxycarbonylphenyl boronic acid 12 (54 mg). Yield: 110 mg (71%), as a yellow product, mp 240-242 ^{o}C.^{1}H NMR (DMSO-d_6): δ 7.95 (dd, 2H, J = 7.8 Hz, 2.1 Hz, 2'-H_{arom} + 6'-H_{arom}), 7.87 (dd, 2H, J = 8.0 Hz, 2.0 Hz, 3'-H_{arom} + 5'-H_{arom}), 7.86 (br s., 2H, 2"-$H_{arom\text{-}OEt}$ + 6"-$H_{arom\text{-}OEt}$), 7.04 (m, 3H, 4-H_{indol} + 3"-H_{arom} + 5"-H_{arom}), 6.93 (d. 1H, J = 8.3 Hz, 7-H_{indol}), 6.73 (dd, 1H, J = 8.3, 2.3 Hz, 6-H_{indol}), 4.10 (q, 2H, J = 7.1 Hz, CH_2CH_3), 3.76 (s, 3H, OMe), 3.34 (s, 2H, CH_2), 1.20 (t, 3H, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 174.3 (CO_2H), 167.8 (C=O + CO_2Et), 157.9 (*C*-OMe), 155.5 (*C*-CO_2Et), 145.2 (C4'$_{arom}$), 137.6 (C3a$_{indol}$), 135.3 (C2$_{indol}$), 134.0 (C1"$_{arom\text{-}CO2Et}$), 131.2, 130.5, 130.1, 129.1, 128.5, 127.7 (C$_{arom}$), 114.5 (C3$_{indol}$ + C3" + C5"$_{arom\text{-}CO2Et}$), 112.7 (C7$_{indol}$), 111.4 (C6$_{indol}$), 101.5 (C4$_{indol}$), 60.3 (OCH_2CH$_3$), 55.3 (OMe), 29.3 (CH_2), 14.1 (OCH$_2$$CH_3$), 13.2 (Me). Anal. calcd for $C_{27}H_{25}NO_5$ (443.49): C, 73.12; H, 5.68; N, 3.16. Found: C, 72.89; H, 5.58; N, 2.89.

2-(1-(4'-Hydroxy)-[1,1'-biphenyl]4-carbonyl)-5-methoxy-2-methyl-indol-3-yl)aceticacid (25):

From 4-hydroxyphenylboronic acid 13 (39 mg). Yield: 121 mg (87%), as a brown powder, mp 218-220 ^{o}C.^{1}H NMR (DMSO-d_6): δ 10.43 (s, 1H, CO_2H), 7.89 (dd, 2H, J = 8.4, 1.8 Hz, 2'-H_{arom} + 6'-H_{arom}), 7.61 (dd, 2H, J = 8.1, 2.0 Hz, 2"-$H_{arom\text{-}OH}$ +6"-$H_{arom\text{-}OH}$), 7.31 (dd, 2H, J = 8.4, 1.8 Hz, 3'-H_{arom} + 5'-H_{arom}), 7.05 (dd, 2H, J = 8.1, 2.0 Hz, 3"-$H_{arom\text{-}OH}$ + 5"-$H_{arom\text{-}OH}$), 6.96 (d, 1H, J = 2.5 Hz, 4-H_{indol}), 6.82 (d, 1H, J = 8.6 Hz, 7-H_{indol}), 6.55 (dd, 1H, J = 8.6, 2.5 Hz, 6-H_{indol}), 3.70 (s, 3H, OMe), 3.45 (s, 2H, CH_2), 2.27 (s, 3H, Me). ^{13}C NMR (DMSO-d_6): δ 176.0 (CO_2H), 168.9 (C=O), 161.2 (C4"-OH), 156.7 (*C*-OMe), 146.8 (C4'$_{arom}$), 138.9 (C3a$_{indol}$), 133.4 (C2$_{indol}$), 132.3, 130.8, 130.1, 129.5, 127.0 (C$_{arom}$), 116.9, 116.6 (C3"$_{arom\text{-}OH}$ + C5"$_{arom\text{-}OH}$), 115.6 (C3$_{indol}$ + C7$_{indol}$), 110.2 (C6$_{indol}$), 101.6 (C4$_{indol}$), 55.2 (OMe), 30.8 (CH_2), 11.6 (Me). Anal. calcd for $C_{25}H_{21}NO_5$ (415.44): C, 72.28; H, 5.10; N, 3.37. Found: C, 71.97; H, 4.99; N, 3.12.

2-(1-(3'-Cyano-[1,1'-biphenyl]4-carbonyl)-5-methoxy-2-methyl-indol-3-yl)aceticacid (26):

From 3-cyanophenylboronic acid 14 (41 mg). Yield: 119 mg (84%), as a yellow powder, mp 228-230 ^{o}C.^{1}H NMR (DMSO-d_6): δ 10.41 (s, 1H, CO_2H), 8.03-7.88 (m, 4H, 2'-H_{arom} + 6'-H_{arom} + 4"-$H_{arom\text{-}CN}$ + 6"-$H_{arom\text{-}CN}$), 7.60 (dd, 2H, J = 8.1, 2.1 Hz, 3'-H_{arom} + 5'-H_{arom}), 7.31 (m, 2H, 3"-$H_{arom\text{-}CN}$ + 5"-$H_{arom\text{-}CN}$), 7.05, 7.04 (2xd, 2H, J = 2.2 Hz, 7-H_{indol} + 4-H_{indol}), 6.55 (dd, 1H, J = 8.3, 2.2 Hz, 6-H_{indol}), 3.70 (s, 3H, OMe), 3.45 (s, 2H, CH_2), 2.27 (s, 3H, Me). ^{13}C NMR (DMSO-d_6): δ 176.0 (CO_2H), 168.9 (C=O), 161.6 (*C*-OMe), 152.4 (C4'$_{arom}$), 144.6 (C1"$_{arom\text{-}CN}$), 138.8 (C3a$_{indol}$), 133.4 (C2$_{indol}$), 132.3, 131.4, 130.8, 129.5, 128.7, 126.9 (C$_{arom}$), 117.0 (CN), 112.3 (C3$_{indol}$ + C3"$_{arom}$), 110.2 (C7$_{indol}$), 108.6 (C6$_{indol}$), 100.8 (C4$_{indol}$), 55.2 (OMe), 30.7 (CH2), 11.6 (Me). Anal. calcd for $C_{26}H_{20}N_2O4$ (424.45): C, 73.57; H, 4.75; N, 6.60. Found: C, 73.32; H, 4.65; N, 6.41.

2-(5-Methoxy-2-mehyl-1-(4'-(trifluoromethyl)-[1,1'-biphenyl]4-carbonyl)-indol-3-yl)aceticacid (27):

From 4-trifluoromeyhylphenylboronic acid 15 (53 mg). Yield: 135 mg (88%), as a yellow powder, mp 218-220°C. ^{1}H NMR (DMSO-d_6): δ 10.42 (s, 1H, CO2H), 7.94 (dd, 1H, J = 7.9, 2.1 Hz, 5'-H_{arom}), 7.89 (dd, 2H, J = 8.1, 2.2 Hz, 2'-H_{arom} + 6'-H_{arom}), 7.66 (dd, 1H, J = 7.9, 2.1 Hz, 3'-H_{arom}), 7.43 (dd, 2H, J = 7.8, 2.5 Hz, 3"-$H_{arom\text{-}CF3}$ + 5"-$H_{arom\text{-}CF3}$), 7.30 (dd, 2H, J = 7.8, 2.5 Hz, 2"-H_{arom} + 6"-H_{arom}), 7.06 (d, 1H, J = 8.2 Hz, 7-H_{indol}), 6.95 (d, 1H, J = 2.2 Hz, 4-H_{indol}), 6.54 (dd, 1H, J = 8.2, 2.1 Hz, 6-H_{indol}), 3.70 (s, 3H, OMe), 3.43 (s, 2H, CH_2), 2.27(s, 3H, Me). ^{13}C NMR (DMSO-d_6): δ 176.1 (CO_2H), 168.9 (C=O), 154.6 (*C*-OMe), 146.0 (C4'$_{arom}$), 143.9 (C1"$_{arom\text{-}CF3}$), 138.9 (C3a$_{indol}$), 133.4 (C2$_{indol}$), 132.3, 130.8, 130.1, 129.5,

127.0, 125.8, 123.0 (C_{arom}), 113.2 ($C3_{indol}$), 110.2 ($C7_{indol}$), 100.8 ($C4_{indol}$), 55.2 (OMe), 30.7 (CH2), 11.6 (Me). Anal. calcd for $C_{26}H_{20}F_3NO_4$ (467.44): C, 66.81; H, 4.31; N, 3.00. Found: C, 66.59; H, 4.27; N, 2.78.

2-(1-4-(5-Formylthiophen-2-yl)benzoyl)-5-methoxy-2-methyl-indol-3-yl)acetic acid (28):
From 5-formyl-2-thiopheneboronic acid 16 (44 mg). Yield: 129 mg (89%), as a brown powder, mp 208-210°C. ^{1}H NMR (DMSO-d_6): δ 10.42 (s, 1H, CO_2H), 9.80 (s, 1H, CHO), 7.88 (dd, 2H, J = 8.1, 2.2 Hz, 2'-H_{arom} + 6'-H_{arom}), 7.64-7.54 (m, 3H, 3'-H_{arom} + 5'-H_{arom} + 5-$H_{thiophen}$), 7.31 (d, 1H, J = 6.7 Hz, 4-$H_{thiophen}$), 7.04 (d, 1H, J = 8.0 Hz, 7-H_{indol}), 6.95 (d, 1H, J = 2.1 Hz, 4-H_{indol}), 6.54 (dd, 1H, J = 8.0, 2.2 Hz, 6-H_{indol}), 3.70 (s, 3H, OMe), 3.43 (s, 2H, CH_2), 2.27 (s, 3H, Me). ^{13}C NMR (DMSO-d_6): δ 182.8 (CHO), 176.0 (CO_2H), 167.2 (C=O), 152.4 (*C*-OMe), 148.3 ($C2''_{thiophen}$), 142.1 ($C5''_{thiophen}$), 139.0 ($C4'_{arom}$), 138.2 ($C4''_{thiophen}$), 136.3 ($C2_{indol}$), 133.4, 132.3, 131.4, 130.8, 130.1, 129.5, 128.6 (C_{arom}), 114.7 ($C(3)_{indol}$), 110.8 ($C6_{indol}$ + $C7_{indol}$), 100.8 ($C4_{indol}$), 55.2 (OMe), 28.7 (CH_2), 11.6 (Me). Anal. calcd for $C_{24}H_{19}NO_6S$ (433.48): C, 66.50; H, 4.42; N, 3.23. Found: C, 66.29; H, 4.35; N, 3.01.

General procedure of preparation of indomethacin having triaryl groups *via* Suzuki-Miyaura cross-coupling reaction (29-31).The compounds were prepared by following the Suzuki reaction procedure described in method A, using instead 2 mol. equiv. of arylboronic acid (0.56 mmol), $Pd(OAc)_2$ (38 mg, 0.17 mmol) and PPh_3 (132 mg, 0.50 mml).

2-(5-Methoxy-2-mehyl-1-(4'-nitro-[1,1'-biphenyl]-4-carbonyl)-indol-3-yl)-1-(4-nitrophenyl)ethanone (29):
From 4-nitrophenylboronic acid 11 (94 mg). Yield: 106 mg (69%), as a brown powder, mp 242-245°C. ^{1}H NMR (DMSO-d_6): δ^1H NMR (DMSO-d_6): δ 8.36-8.27 (m, 6H, H_{arom}), 7.87-7.13 (m, 6H, H_{arom}), 7.01 (d, 1H, J = 8.2 Hz, 7-H_{indol}), 6.92 (d, 1H, J = 2.3 Hz, 4-H_{indol}), 6.60 (dd, 1H, J = 2.3, 8.3 Hz, 6-H_{indol}), 3.72 (s, 3H, OMe), 3.46 (s, 2H, CH_2), 2.25 (s, 3H, Me). ^{13}C NMR (DMSO-d_6): δ 195.2 (CH_2CO), 168.5 (C=O), 159.3 (*C*-OMe), 151.3, 147.1 (2xC-NO_2), 146.5 ($C4'_{arom}$ + $C1''_{arom}$), 140.0 (C_{arom}), 136.1 ($C3a_{indol}$), 133.2 ($C2_{indol}$), 131.6, 130.8, 130.4, 129.0, 128.8, 128.5, 127.9, 124.0 (C_{arom}), 113.0 (C-6_{indol} + $C7_{indol}$), 108.2 ($C3_{indol}$), 100.2 ($C4_{indol}$), 55.6 (OMe), 33.9 (CH2), 12.4 (Me).Anal. calcd for $C_{31}H_{23}N_3O_7$ (549.53): C, 67.75; H, 4.22; N, 7.65. Found: C, 67.54; H, 4.14; N, 7.41.

2-(1-(4'-Hydroxy-[1,1'-biphenyl]-4-carbonyl)-5-methoxy1-2-methyl-indol-3-yl)-1-(4 hydroxyphenyl)ethanone (30):
From 4-hydroxyphenylboronic acid 13 (78 mg). Yield: 90 mg (65%), as a pale brown powder, mp 232-235°C. ^{1}H NMR (DMSO-d_6): δ7.91 (dd, 2H, J = 8.5, 2.1 Hz, 2'-H_{arom} + 6'-H_{arom}), 7.73 (m, 4H, 2-$H_{arom\text{-}OH}$ +6-$H_{arom\text{-}OH}$), 7.69 (dd, 2H, J = 8.1, 2.0 Hz, 2''-$H_{arom\text{-}OH}$ +6''-$H_{arom\text{-}OH}$), 7.31-7.11 (m, 4H, 3-$H_{arom\text{-}OH}$ +5-$H_{arom\text{-}OH}$ + 3''-$H_{arom\text{-}OH}$ +5''-$H_{arom\text{-}OH}$), 6.98 (d, 1H, J = 8.0 Hz, 7-H_{indol}), 6.94 (d, 1H, J = 2.2 Hz, 4-H_{indol}), 6.63 (dd, 1H, J = 8.0, 2.2 Hz, 6-H_{indol}), 3.71 (s, 3H, OMe), 3.44 (s, 2H, CH_2), 2.23 (s, 3H, Me). ^{13}C NMR (DMSO-d_6): δ 194.9 (CH_2CO), 168.2 (C=O), 161.2 (C_{arom}-OH), 159.3, 159.1 (*C*-OMe + $C4''_{arom}$-OH), 145.5 ($C4'_{arom}$), 136.8 ($C3a_{indol}$), 133.9 ($C2_{indol}$), 133.0, 131.6, 130.8, 130.4, 128.8, 128.5, 127.9, 115.8, 114.9 (C_{arom}), 112.9 (C-6_{indol} + $C7_{indol}$), 106.9 ($C3_{indol}$), 100.1 ($C4_{indol}$), 55.5 (OMe), 34.2 (CH2), 12.7 (Me). Anal. calcd for $C_{31}H_{23}N_3O_7$ (491.18): C, 75.75; H, 5.13; N, 2.85. Found: C, 75.54; H, 5.02; N, 2.69.

4'(3-(2-(3-Cyanophenyl)-2-oxoethyl)-5-methoxy-2-methyl-indole-1-carbonyl)-1,1'-biphenyl]-3-carbonitrile (31):
From 3-cyanophenylboronic acid 14 (83 mg). Yield: 102 mg (71%), as a pale yellow powder, mp 239-242°C. ^{1}H NMR (DMSO-d_6): δ8.24 (d, 1H, J = 2.1 Hz, $C2_{arom\text{-}CN}$), 8.11 (m, 1H,

$C6_{arom-CN}$), 8.07-7.46 (m, 10H, H_{arom}), 7.12, (d, 1H, *J* = 8.0 Hz, 7-H_{indol}), 6.93 (d, 1H, *J* = 2.0 Hz, 4-H_{indol}), 6.69 (dd, 1H, *J* = 8.0, 2.0 Hz, 6-H_{indol}), 3.75 (s, 3H, OMe), 3.51 (s, 2H, CH_2), 2.24 (s, 3H, Me).[13]C NMR (DMSO-d_6): *δ* 195.6 (CH_2*CO*), 168.1 (C=O), 160.8 (*C*-OMe), 149.0($C4'_{arom}$), 143.9 ($C1''_{arom-CN}$), 138.2 ($C3a_{indol}$), 136.2 ($C1_{arom-CN}$), 135.1 ($C4_{arom-CN}$), 133.1 ($C2_{indol}$), 132.0, 131.4, 130.5, 130.0, 129.1, 128.5, 128.2, 127.9 (C_{arom}), 117.8 (CN), 112.9 ($C3''_{arom-CN}$ + $C7_{indol}$), 112.1 ($C6_{indol}$ + $C3_{arom-CN}$), 106.4 ($C3_{indol}$), 100.1 ($C4_{indol}$), 55.4 (OMe), 34.5 (CH2), 12.5 (Me). Anal. calcd for $C_{33}H_{23}N_3O_3$ (509.55): C, 77.78; H, 4.55; N, 8.75. Found: C, 77.53; H, 4.41; N, 8.02.

Methyl2-(3-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetyl)thioureido)carboxylic acid esters (33-35):

A solution of 4 (200 mg, 0.53 mmol) and NH_4NCS (40 mg, 0.53 mmol) in dry acetone (15 mL) was heated under reflux for 1h. After cooling the solution was filtered, the desired amino acid esters hydrochloride in dry acetone (15 mL) were added rapidly on the above solution and vigorous stirring and reflux for 6 h (monitored by TLC, eluent: $CHCl_3$-MeOH 95:5). After cooling, an excess of crushed ice was poured on the mixture with vigorous stirring. The resulting compound was collected, washed with acetone and recrystallized twice from EtOH.

Methyl2-(3-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetyl) thioureido)acetate (33):

From L-alanine methyl ester hydrochloride (74 mg, 0.53 mmol). Yield: 194 mg (75%), as a yellow powder, mp 81-82°C, R_f = 0.72. [1]H NMR (DMSO-d_6): *δ* 10.82 (s, 1H, NH), 7.82 (dd, 2H, *J* = 8.1, 1.9 Hz, 2'-H_{arom} + 6'-H_{arom}), 7.54 (dd, 2H, *J* = 8.1, 1.9 Hz, 3'-H_{arom} + 5'-H_{arom}), 7.36 (d, 1H, *J* = 8.1 Hz, 7-H_{indol}), 7.14 (d, 1H, *J* = 1.8 Hz, 4-H_{indol}), 6.64 (dd, 1H, *J* = 8.1, 1.8 Hz, 6-H_{indol}), 3.83, 3.81 (2xs, 6H, CH_2-8 + OMe), 3.69 (s, 3H, CO_2Me), 3.44 (br s., 1H, 13-H), 2.34 (s, 3H, C(2)-Me), 1.23 (s, 3H, C(13)-Me). [13]C NMR (DMSO-d_6): *δ* 187.0 (C=S), 173.3 (*CO*$_2$Me), 171.0 (C(9)=O), 167.7 (C=O), 155.5 (*C*-OMe), 139.1 (C-Cl), 137.7 ($C3a_{indol}$), 132.5 ($C2_{indol}$), 130.8, 130.5, 129.8, 128.9, 127.0 (C_{arom}), 111.5 ($C6_{indol}$ + $C7_{indol}$), 106.4 (C-3), 101.5 ($C4_{indol}$), 58.3 (C-13), 55.7 (OMe), 51.9 (CO_2*Me*), 29.4 (C-8), 17.3 (C(13)-*Me*), 12.8 (C2-*Me*). Anal. calcd for $C_{24}H_{24}ClN_3O_5S$ (501.98): C, 57.42; H, 4.82; N, 8.37. Found: C, 57.19; H, 4.76; N, 8.11.

Methy2-(3-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-yl)acetyl) thioureido)butanoate (34):

From L-valine methyl ester hydrochloride (89 mg, 0.53 mmol). Yield: 244 mg (87%), as a green powder, mp 218-220°C, R_f = 0.78. [1]H NMR (DMSO-d_6): *δ* 10.60 (s, 1H, NH), 10.02 (s, 1H, NH), 7.90 (dd, 2H, *J* = 8.1, 2.3 Hz, 2'-H_{arom} + 6'-H_{arom}), 7.50 (dd, 2H, *J* = 8.1, 2.3 Hz, 3'-H_{arom} + 5'-H_{arom}), 7.33 (d, 1H, *J* = 7.9 Hz, 7-H_{indol}), 7.18 (d, 1H, *J* = 2.0 Hz, 4-H_{indol}), 6.67 (dd, 1H, *J* = 7.9, 2.0 Hz, 6-H_{indol}), 3.85 (s, 3H, OMe), 3.74 (s, 2H, CH_2-8), 3.64 (s, 3H, CO_2Me), 3.24 (dd, 1H, *J* = 8.5, 5.0 Hz, 13-H), 2.37 (s, 3H, C(2)-Me), 1.23 (s, 3H, C(13)-Me), 1.84 (m, 1H, 14-H), 1.07 (d, 6H, *J* = 6.5 Hz, 2xMe).[13]C NMR (DMSO-d_6): *δ* 186.8 (C=S), 172.3 (C(9)=O), 170.5 (*CO*$_2$Me), 167.8 (C=O), 155.3 (*C*-OMe), 140.3 (C-Cl), 137.6 ($C3a_{indol}$), 135.5 ($C2_{indol}$), 131.1, 130.5, 130.1, 129.1 (C_{arom}), 112.8 ($C7_{indol}$), 111.4 ($C6_{indol}$), 107.3 (C-3), 101.6 ($C4_{indol}$), 60.4 (C-13), 55.4 (OMe), 51.1 (CO_2*Me*), 30.7 (C-8), 29.3 (C-14), 18.3 (2xMe), 13.2 (C(2)-*Me*). Anal. calcd for $C_{26}H_{28}ClN_3O_5S$ (530.04): C, 58.92; H, 5.32; N, 7.93. Found: C, 58.73; H, 5.22; N, 7.69.

Methyl2-(3-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-yl)acetyl)thioureido)-3-mercapto propanoate (35):

From cysteine methyl ester hydrochloride (91 mg, 0.53 mmol). Yield (0.19 g) (95%), as a yellow powder, mp168-170°C, R_f = 0.72. [1]H NMR (DMSO-d_6): *δ* 9.91 (s, 1H, NH), 9.64 (s,

1H, NH), 7.75 (dd, 2H, *J* = 7.8, 2.0 Hz, 2'-H_{arom} + 6'-H_{arom}), 7.56 (dd, 2H, *J* = 7.8, 2.03 Hz, 3'-H_{arom} + 5'-H_{arom}), 7.25 (d, 1H, *J* = 8.0 Hz, 7-H_{indol}), 7.21 (d, 1H, *J* = 2.0 Hz, 4-H_{indol}), 6.92 (dd, 1H, *J* = 8.0, 2.1 Hz, 6-H_{indol}), 3.77 (s, 3H, OMe), 3.75 (m, 3H, CH_2-8 + 13-H), 3.67 (s, 3H, CO_2*Me*), 3.46 (m, 2H, *CH_2*-SH), 2.27 (s, 3H, C(2)-Me).^{13}C NMR (DMSO-d_6): *δ* 186.2 (C=S), 173.0 (C(9)=O), 171.9 (*CO_2*Me), 167.1 (C=O), 151.1 (*C*-OMe), 139.3 (C-Cl), 136.6 (C3a_{indol}), 136.2 (C2$_{indol}$), 132.4, 130.9, 129.4, 129.2, 128.4 (C_{arom}), 112.8 (C7$_{indol}$), 112.5 (C7$_{indol}$), 110.6 (C6$_{indol}$), 109.3 (C-3), 99.4 (C4$_{indol}$), 69.6 (C-13), 55.3 (OMe), 51.7 (CO_2*Me*), 30.6 (C-8), 26.4 (CH_2-SH), 13.2 (C2-*Me*). Anal. calcd for $C_{24}H_{24}ClN_3O_5$ (535.05): C, 53.98; H, 4.43; N, 7.87. Found: C, 53.77; H, 4.32; N, 7.58.

General procedure for the preparation of methyl 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetamido carboxylate derivatives (36-41). To a cold solution of amino acid esters hydrochloride (0.28 mmol) at - 5°C in MeCN (20 mL), indomethacin 1 (100 mg, 0.28 mmol), HOBT (38 mg, 0.28 mmol) and DCC (58 mg, 0.28 mmol) were added successively. The reaction mixture was stirred at 0°C for 1 h, 5°C for 1h, and 23°C for 16 h. Dicyclohexylurea (DCU) was filtered and the filterate was evaporated to dryness. The residue was stirred in ethyl acetate (20 mL), filtered, washed successively with saturated (NaCl) solution (20 mL), 5% $NaHCO_3$ solution (20 mL), 1.0 mol L^{-1} HCl (10 mL), and finally washed with water. The organic layer was dried by (Na_2SO_4), filtered, and the filtrated was evaporated to dryness and recrystallized from EtOH.

Methyl2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-yl)acetamido acetate (36):
From glycine methyl ester hydrochloride (35 mg). Yield: 84 mg (70%), as a brown powder, mp 68-70°C, R_f = 0.48. ^{1}H NMR (DMSO-d_6): *δ* 8.50 (d, 1H, $J_{NH,H11}$ = 5.5 Hz, NH), 7.71-7.62 (m, 4H, H_{arom}), 7.15 (d, 1H, *J* = 8.0 Hz, 7-H_{indol}), 6.94 (br s., 2H, *J* = 2.0 Hz, 4-H_{indol} + 6-H_{indol}), 3.78 (d, 2H, *J* = 2.2 Hz, CH_2-11), 3.75 (br s., 5H, CH_2-8 + OMe), 3.65 (s, 3H, CO_2Me), 2.23 (s, 3H, C(2)-Me). ^{13}C NMR (DMSO-d_6): *δ*173.0 (*CO_2*Me), 170.1 (C(9)=O), 167.7 (C=O), 156.6 (*C*-OMe), 137.1 (C-Cl), 135.2 (C3a_{indol} + C-2), 131.1, 130.9, 129.0 (C_{arom}), 113.8 (C7$_{indol}$), 110.6 (C6$_{indol}$), 106.1 (C-3), 101.9 (C4$_{indol}$), 55.5 (OMe), 53.6 (CO_2*Me*), 43.5 (C-11), 30.7 (C-8), 14.0 (C2-*Me*).Anal. calcd for $C_{22}H_{21}ClN_2O_5$ (428.87): C, 61.61; H, 4.94; N, 6.53. Found: C, 61.48; H, 4.79; N, 6.33.

Methyl2-(1-(4-chorobenzoyl)-5-methoxy-2-methyl-indol-3-yl)acetamidopropanoate(37):
From L-alanine methyl ester hydrochloride (39 mg): Yield: 86 mg (65%), as a semi-solid, R_f = 0.68. ^{1}H NMR (DMSO-d_6): *δ* 8.53 (d, 1H, $J_{NH,H11}$ = 5.8 Hz, NH), 7.66 (br s., 4H, H_{arom}), 7.08 (d, 1H, *J* = 7.9 Hz, 7-H_{indol}), 6.94 (br s., 2H, 4-H_{indol} + 6-H_{indol}), 4.18 (dd, 2H, *J* = 7.2, 5.8 Hz, 11-H), 3.75 (s, 5H, CO_2Me + OMe), 3.65 (s, 3H, CH_2-8), 2.30 (s, 3H, C2-Me), 1.70 (d, 3H, *J* = 7.1 Hz, C11-Me). ^{13}C NMR (DMSO-d_6): *δ*173.2 (*CO_2*Me), 171.6 (C9=O), 167.7 (C=O), 156.7 (*C*-OMe), 137.2 (C-Cl), 134.6 (C3a_{indol}), 133.2 (C-2), 131.9, 130.9, 128.9 (C_{arom}), 112.6 (C7$_{indol}$), 110.7 (C(6)$_{indol}$), 105.5 (C-3), 102.4 (C4$_{indol}$), 57.5 (OMe), 55.3 (C-11), 53.2 (CO_2*Me*), 29.9 (C-8), 18.2 (C11-*Me*), 13.3 (C2-*Me*).Anal. calcd for $C_{23}H_{23}ClN_2O_5$ (442.89): C, 62.37; H, 5.23; N, 6.33. Found: C, 62.09; H, 5.11; N, 6.20.

Methyl2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-yl)acetamido)-3-hydroxy)propanoate (38):
From L-serine methyl ester hydrochloride (43 mg). Yield: 104 mg (78%), as a brown powder, mp 78-80°C, R_f = 0.50. ^{1}H NMR (DMSO-d_6): *δ* 8.38 (br s., 1H, NH), 7.64 (br s., 4H, H_{arom}), 7.06 (br s., 2H,4-H_{indol} + 7-H_{indol}), 6.93 (d., 1H, *J* = 7.8 Hz, 6-H_{indol}), 4.20 (dd, 1H, *J* = 8.3, 4.8 Hz, 11-H), 3.78 (s, 5H, CO_2*Me*), 3.59 (s, 3H, OMe), 3.57 (s, 2H, CH_2-8), 3.58 (t, 1H, *J* = 5.7 Hz, OH), 3.40 (s, 3H, CH_2OH), 2.31 (s, 3H, C(2)-Me). ^{13}C NMR (DMSO-d_6): *δ*172.4 (*CO_2*Me), 170.2 (C9=O), 167.8 (C=O), 156.7 (*C*-OMe), 137.5 (C-Cl), 135.1(C3a_{indol} + C-2),

131.7, 131.1, 130.1, 129.0 (C_{arom}), 112.4 ($C7_{indol}$), 111.5 ($C6_{indol}$), 104.4 (C-3), 102.0 ($C4_{indol}$), 59.7 (C-11), 55.4 (OMe), 53.5 (CO_2*Me*), 31.3 (C-8), 26.2 (CH_2-OH), 13.9 (C2-*Me*).Anal. calcd for $C_{23}H_{23}ClN_2O_5S$ (474.96): C, 58.16; H, 4.88; N, 5.90. Found: C, 57.92; H, 4.69; N, 5.69.

Methyl2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-yl)acetamido)-3-methyl butanoate(39):

From L-valine methyl ester hydrochloride (47 mg). Yield: 94 mg (71%), as a light-yellow powder, mp 110-113°C, R_f = 0.63. ^{1}H NMR (DMSO-d_6): δ 8.61 (d, 1H, $J_{NH,H11}$ = 5.6 Hz, NH), 7.69 (br s., 4H, H_{arom}), 7.12 (d, 1H, J = 8.1 Hz, 7-H_{indol}), 7.01 (br s., 2H, 4-H_{indol} + 6-H_{indol}), 4.20 (dd, 2H, J = 7.4, 5.6 Hz, 11-H), 3.77 (s, 5H, CO_2Me), 3.72 (s, 3H, OMe), 3.65 (s, 3H, CH_2-8), 2.28 (s, 3H, C(2)-Me), 2.07 (m, 1H, 12-H), 0.81, 0.83 (2s, 6H, 2xMe). ^{13}C NMR (DMSO-d_6): δ172.8 (*C*O_2Me), 170.9 (C(9)=O), 167.1 (C=O), 156.5 (*C*-OMe), 137.0 (C-Cl), 134.7 ($C3a_{indol}$), 133.5 (C-2), 131.6, 130.3, 128.6, 128.3 (C_{arom}), 112.8 ($C7_{indol}$), 110.5 ($C6_{indol}$), 105.3 (C-3), 102.5 ($C4_{indol}$), 57.5 (OMe), 62.1 (C-11), 52.4 (CO_2*Me*), 31.1 (C-8), 30.5 (C-12), 18.9, 17.6 (2xMe), 13.1 (C2-*Me*).Anal. calcd for $C_{25}H_{27}ClN_2O_5$ (470.95): C, 63.76; H, 5.78; N, 5.95. Found: C, 63.53; H, 5.69; N, 5.81.

Methyl2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-yl)acetamido)-4-methyl pentanoate (40):

From L-leucine methyl ester hydrochloride (51 mg). Yield: 87 mg (64%), as a colorless powder, mp 131-134°C, R_f = 0.68. ^{1}H NMR (DMSO-d_6): δ 8.63 (d, 1H, $J_{NH,H11}$ = 5.5 Hz, NH), 7.70 (br s., 4H, H_{arom}), 7.12 (d, 1H, J = 7.9 Hz, 7-H_{indol}), 6.98 (br s., 2H, 4-H_{indol} + 6-H_{indol}), 4.26 (dd, 2H, J = 7.5, 5.5 Hz, 11-H), 3.78 (s, 3H, CO_2Me), 2.74 (s, 3H, OMe), 4.41 (m, 1H, 11-H), 3.70 (s, 3H, CH_2-8), 2.05 (m, 2H, CH_2-12), 1.17 (m, 1H, 13-H), 0.80, 0.82 (2s, 6H, 2xMe). ^{13}C NMR (DMSO-d_6): δ173.0 (*C*O_2Me), 171.2 (C9=O), 167.5 (C=O), 156.5 (*C*-OMe), 136.9 (C-Cl), 134.2 ($C3a_{indol}$), 133.0 (C-2), 131.7, 130.3, 129.9, 128.1 (C_{arom}), 112.9 ($C7_{indol}$), 110.5 ($C6_{indol}$), 105.2 (C-3), 102.1 ($C4_{indol}$), 57.3 (OMe), 56.1 (C-11), 53.8 (CO_2*Me*), 51.1 (C-12), 32.0 (C-8), 30.8 (C-13), 13.1 (C2-*Me*).Anal. calcd for $C_{26}H_{29}ClN_2O_5$ (484.97): C, 64.39; H, 6.03; N, 5.78. Found: C, 64.18; H, 5.97; N, 5.62.

Methyl6-amino-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-yl)acetamido)-3-hexanoate (41):

From L-lysine methyl ester hydrochloride (55 mg, 0.28 mmol). Yield: 111 mg (79%), as a light yellow powder, mp98-102°C, R_f = 0.28. ^{1}H NMR (DMSO-d_6): δ 8.50 (d, 1H, J = 5.7 Hz, NH), 7.72- 7.64 ((m, 4H, H_{arom}), 7.08 (d., 1H, J = 7.5 Hz, 7-H_{indol}), 6.96, 6.95 (2xbr s., 2H, 4-H_{indol} + 6-H_{indol}), 5.96 (d, 2H, J = 8.1 Hz, NH_2), 4.50 (dd, 1H, J = 8.2, 5.7 Hz, 11-H), 3.78 (s, 3H, OMe), 3.75 (s, 3H, CO_2*Me*), 3.54 (s, 2H, CH_2-8), 2.51 (m., 2H, CH_2-15), 2.23 (s, 3H, C(2)-Me), 1.71 (m, 4H, CH_2-12 + CH_2-14), 1.60 (m, 2H, CH_2-13). ^{13}C NMR (DMSO-d_6): δ 173.0 (*C*O_2Me), 170.0 (C9=O), 167.8 (C=O), 156.6 (*C*-OMe), 137.5 (C-Cl), 135.2 ($C3a_{indol}$), 134.2 (C-2), 132.0,130.9, 130.8, 128.9 (C_{arom}), 112.6 ($C7_{indol}$), 110.6 ($C6_{indol}$), 102.5 (C-3), 101.9 ($C4_{indol}$), 56.2 (C-11), 55.4 (OMe), 53.1 (CO_2*Me*), 47.5 (C-15),33.3 (C-8), 30.7 (C-12), 28.9 (C-14), 24.4 (C-13), 14.0 (C2-*Me*). Anal. calcd for $C_{26}H_{30}ClN_3O_5$ (499.99): C, 62.46; H, 6.05; N, 8.40. Found: C, 62.27; H, 5.96; N, 8.25.

2.3 Biological Activity

2.3.1 Antiviral assay

2.3.1.1 In vitro anti-HIV assay

Evaluation of the antiviral activity of the new synthesized compounds against HIV-1 strain III_B and HIV-2 strain (ROD) in MT-4 cells was performed using the MTT assay as previously described [28,29]. Briefly, stock solutions (10 x final concentration) of test compounds were added in 25 *µ*L volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on mock-and HIV-infected cells at the beginning of each experiment. Serial 5-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments). Untreated control HIV-and mock-infected cell samples were included for each sample.HIV-1(III_B) [30] or HIV-2 (ROD) [31] stock (50 *µ*L) at 100-300 $CCID_{50}$ (50% cell culture infectious dose) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells in order to assess the cytotoxicity of the test compound. Exponentially growing MT-4 cells [32] were centrifuged for 5 min at 1,000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at 6 x 10^5 cells/mL and 50 *µ*L volumes were transferred to the microtiter tray wells. Five days after infection at ambient temperature, the viability of mock-and HIV-infected cells was examined spectrophotometrically by the MTT assay.

2.3.1.2 Anti-HCV assay

Huh 5.2 cells, containing the hepatitis C virus genotype 1b I389luc-ubi-neo/NS3-3'/5.1 replicon [33] were sub-cultured in DMEM supplemented with 10% FCS, 1% non-essential amino acids, 1% penicillin/streptomycin and 2% Geneticin at a ratio of 1:3 to 1:4, and grown for 3 - 4 days in 75 cm² tissue culture flasks. One day before addition of the compound, cells were harvested and seeded in assay medium (DMEM, 10% FCS, 1% non-essential amino acids, 1% penicillin/streptomycin) at a density of 6500 cells/well (100 µL/well) in 96-well tissue culture microtiter plates for evaluation of anti-metabolic effect and Cultur-Plate (Perkin Elmer) for evaluation of the antiviral effect. The microtiter plates were incubated overnight (37°C, 5% CO_2, 95-99% relative humidity), yielding a non-confluent cell monolayer.

The evaluation of the anti-metabolic as well as antiviral effect of each compound was performed in parallel. Four-step, 1-to-5 compound dilution series were prepared for the first screen, to collect data for a more detailed dose-response curve, an eight-step, 1-to-2 dilution series was used. Following assay setup, the microtiter plates were incubated for 72 hours (37°C, 5% CO_2, 95-99% relative humidity). For the evaluation of anti-metabolic effects, the assay medium was aspirated, replaced with 75 µL of a 5% MTS solution in phenol red-free medium and incubated for 1.5 hours (37°C, 5% CO_2, 95-99 % relative humidity). Absorbance was measured at a wavelength of 498 nm (Safire², Tecan), and optical densities (OD values) were converted to percentage of untreated controls. For the evaluation of antiviral effects, assay medium was aspirated and the cell monolayers were washed with phosphate-buffered saline (PBS). The wash buffer was aspirated, and 25 µL of GloLysis Buffer (Promega) was added allowing for cell lysis to proceed for 5 min at room temperature. Subsequently, 50 µL of Luciferase Assay System (Promega) was added, and the luciferase luminescence signal was quantified immediately (1000 ms integration time/well, Safire², Tecan). Relative luminescence units were converted into percentage of untreated controls.

The EC_{50} and EC_{90} (values calculated from the dose-response curve) represent the concentrations at which 50% and 90% inhibition, respectively, of viral replication is achieved. The CC_{50} (value calculated from the dose-response curve) represents the concentration at which the metabolic activity of the cells is reduced by 50% as compared to untreated cells.

A concentration of compound is considered to elicit a genuine antiviral effect in the HCV replicon system when the anti-replicon effect is well above the 70% threshold at concentrations where no significant anti-metabolic activity is observed [33].

2.3.1.3 Cells and HCV viruses

The Huh-5-2 and Huh 9-13 HCV subgenomic replicon-containing cells were provided by Prof. R. Bartenschlager (University of Heidelberg, Heidelberg, Germany).

2.3.2 Anti-Kinesin Eg5 assay

The ATPase activity of the Eg5 motor domain was measured using the malachite green assay as described earlier [45]. The reactions were performed in reaction buffer (80 mM Pipes, pH 6.8; 1 mMEGTA, 1 mM $MgCl_2$, 0.1 mg/mL^{-1} BSA, 1 mMtaxol) supplemented with Eg5 (48 nM) fusion protein and microtubules (200 nM). Ten min after compound addition, reactions were started by the addition of ATP (50 mM) and incubated at RT for 7 min. The reactions were stopped by adding perchloric acid (444 mM, Fluka), and the colour reaction was started by adding the developer solution (1M HCl (Sigma), 33 mM malachite green (Sigma), 775 mM ammonium molybdatetetrahydrate (Sigma)). After 20 min, the absorbance at 610 nm was measured using a plate reader (Victor 2, Perkin-Elmer). The IC_{50} values were determined in three independent experiments for each compound. Fig. 1 shows the ATPase activity screening assay.

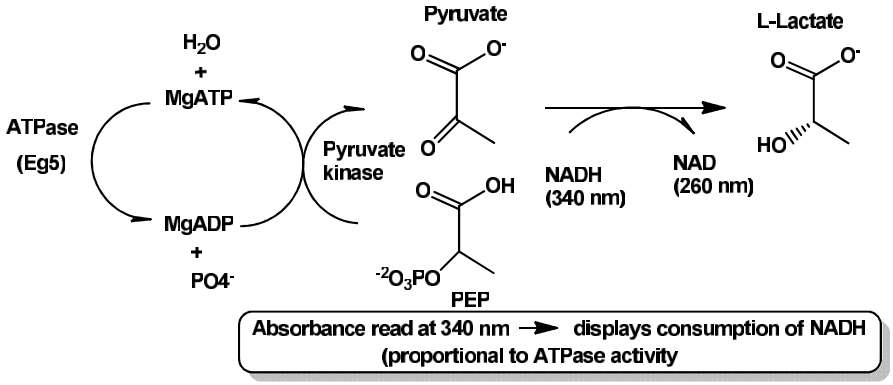


Fig. 1. ATPase activity screening assay

3. RESULTS AND DISCUSSION

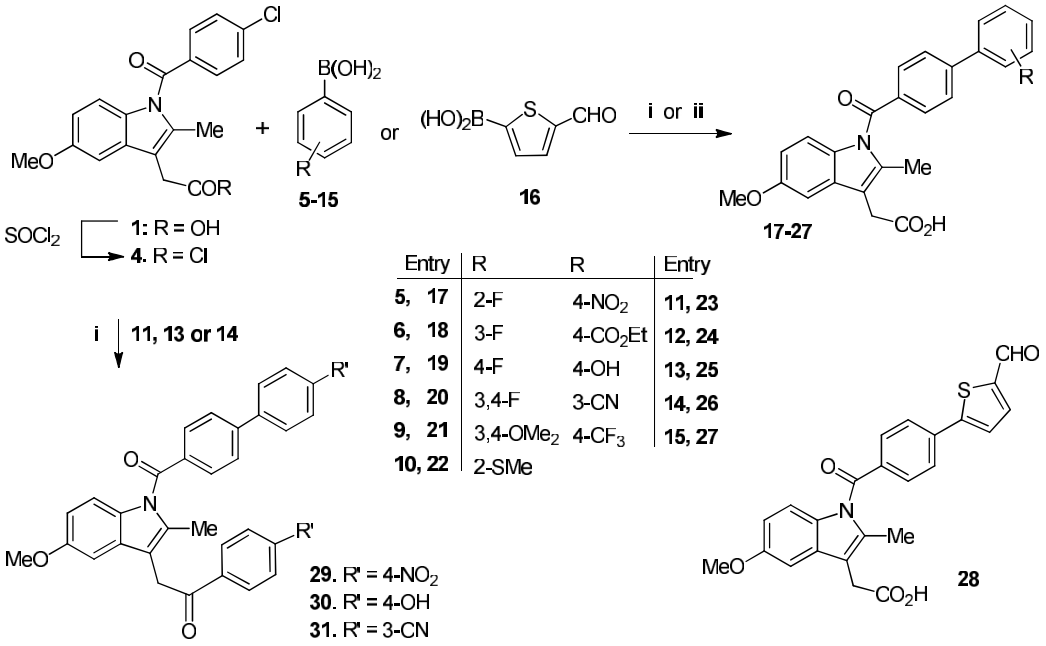
3.1 Chemistry

The Suzuki coupling reaction [34] has been employed in the preparation of new indomethacin analogues. Thus, treatment of indomethacin 1 with the appropriate arylboronic acids (e.g.: 2-fluorophenyl-, 3-fluorophenyl-, 4-fluorophenyl-, 3,4-difluorophenyl-, 3,4-

dimethoxy phenyl-, 2-methylthiophenyl-, 4-nitrophenyl-, 4-ethoxycarbonylphenyl-, 4-hydroxyphenyl-, 3-cyanophenyl-, and 4-triflouromethylphenyl boronic acids 5-15 and 5-formyl-2-thiopheneboronic acid 16, using a mixture of palladium acetate / triphenylphosphine complex and sodium bicarbonate as a catalyst afforded 17-28 in 72-78% yield, respectively. The *N*-heterocyclic carbenecomplexs (Pd-NHC) performed Suzuki cross-coupling reaction at reasonable temperature, where the catalyst palladium 1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene(1,4-naphthoquinone)palladium(0)dimer $[Pd(iPr)(NQ)]_2$ is considered as one of these complexes, exhibited impressive activity in the Suzuki-Miyaura coupling of aryl chlorides with phenyl boronic acids. Accordingly, 1 was treated with aryl boronic acids 5-7 in the presence of 0.5 mole of $[Pd(iPr)(NQ)]_2$ [35], potassium *tert*-butoxide in 2-propanol as a solvent. This method afforded almost better yields of 17-19 (85, 83 and 89%, respectively) (Scheme 1). Interestingly, the triaryl-indomethacin analogues 39-31 were obtained in 69, 65 and 71% yield, respectively, from treatment of 4 [15], prepared from chlorination of 1, and 2 mol. equiv. of arylboronic acids (11, 13 and 14), employing Suzuki-Miyaura cross-coupling reaction (Scheme 1).

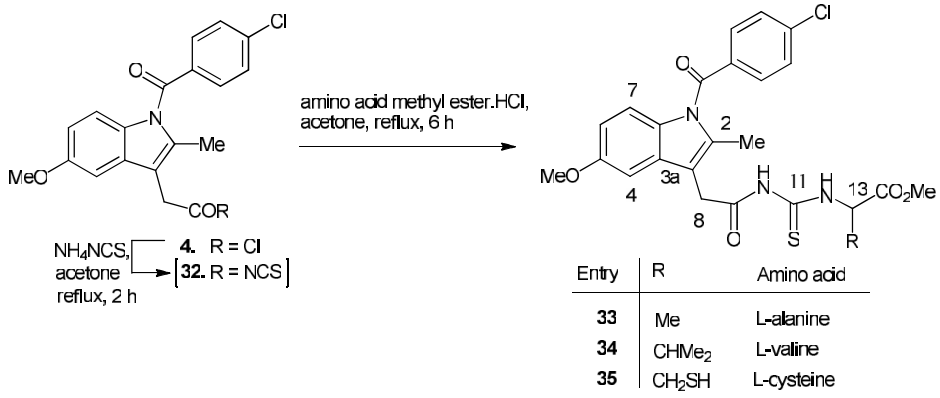
The structures of 17-28 were identified by the ^{1}H and ^{13}C NMR spectra, which are in agreement with the suggested structures, and showed rather similar patterns for the indole scaffold. The 1H NMR spectra were and characterized by the presence of additional aromatic proton and carbon atoms, indicative for arylation of indomethacin 1. The low field singlets at the regions δ 10.43-10.41 ppm were assigned to CO_2H signals. The aromatic protons were appeared as doublets, doublet of doublets, multiplets and broad singlets at the regions δ 8.34-6.55 ppm, whereas H-4 of the indole backbone resonated as doublets at the regions δ 7.05-6.89 ppm (J ~ 2.2 Hz). 6-H and 7-H protons were appeared as doublet of doublets and doublets at the regions δ 6.78-6.53 and δ 7.06-6.82 ppm *(*J ~ 8.2, 2.2 Hz), respectively. The singlets at the regions δ 3.47-3.25 ppm were assigned to the methylene protons (CH_2-8). The other protons of the prepared compounds were fully analyzed (*cf.* Experimental section). In the ^{13}C NMR spectra of 17-28, the CO_2H signals appeared at the regions δ 176.1-174.3 ppm, while the resonances at δ 169.0-167.2 ppm were assigned to C=O carbon atoms. The *C*-OMe(C-5) carbons appeared at the regions δ 161.6-152.4 ppm, and C-3a resonated at the regions δ139.0-133.3 ppm. C-2 and C-3carbons resonated at the regions δ 136.3-132.2 ppm, and δ 116.0-108.7 ppm, respectively, while the resonances at the regions δ101.6-100.0 ppm, δ 111.4-108.3 ppm, and δ115.4-110.2 ppm were assigned to C-4, C-6 and C-7, respectively. Aromatic carbon atoms and carbons of the other substituents were fully assigned (*cf*. Experimental section). The structures of 29-31 were identified from their ^{1}H and ^{13}C NMR spectra, which almost similar for those of the analogues 23, 25 and 26. In the ^{13}C NMR of 29-31, the resonances at δ 195.2, 194.9 and 195.6 ppm were assigned to the carbonyl carbon atoms (CH_2*CO*Ar). The protons and carbon atoms of these compounds were fully analyzed (cf. Experimental section).

Our work was modified by selecting 4, as a precursor for the synthesis of new indomethacin-thioureido amino acid analogues to examine their antiviral activity in comparison to 16-27. Thus, treatment of 4 with ammonium thiocyanate in acetone by following Kabbani approach [36]afforded 32,which was directly treated with the amino acid derivatives (l-alanine, l-valine and l-serine methyl esters hydrochloride) to give, after purification, the thioureido derivatives 33-35 in 75, 87, and 95 % yield, respectively. The synthetic reactions are summarized in scheme 2.



Entry	R	R	Entry
5, 17	2-F	4-NO_2	11, 23
6, 18	3-F	4-CO_2Et	12, 24
7, 19	4-F	4-OH	13, 25
8, 20	3,4-F	3-CN	14, 26
9, 21	3,4-OMe_2	4-CF_3	15, 27
10, 22	2-SMe		

Scheme 1. *Condition and reagents*: (i) $Pd(OAc)_2$, Ph_3P, K_2CO_3, *n*-PrOH, reflux; or (ii) $[Pd(iPr)(NQ)]_2$ complex, K ^{tert}OBu, 2-PrOH, 50°C, 1 h



Entry	R	Amino acid
33	Me	L-alanine
34	$CHMe_2$	L-valine
35	CH_2SH	L-cysteine

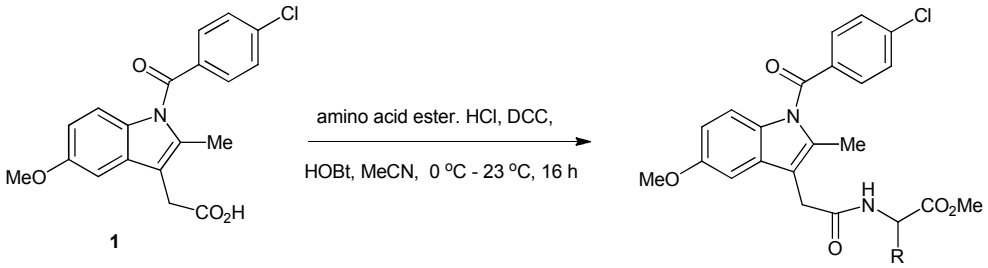
Scheme 2. Synthesis of methyl 2-(3-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-yl)acetyl)thioureido)-carboxylate derivatives

The structures of 33-35 were determined from their NMR (^{1}H, ^{13}C), since they showed a similar pattern of the indol moiety and the aromatic protons (*cf.* Experimental Section). CH_2-8 protons appeared as singlets at δ 3.83, 3.74 and 3.75 ppm, respectively, while H-13 protons signals were resonated at broad singlet, doublet of doublets (J = 8.5, 5.0 Hz) and multiplet at δ 3.44, 3.24 and 3.75 ppm, respectively. The other protons of amino acid esters were fully analyzed. The ^{13}C NMR spectra of 33-35 contained almost similar resonance signals of the indole, aromatic and thioureido carbon atoms. The chemical shifts at δ 187.0, 186.8 and 186.2 ppm were assigned to C=S carbon atoms of the thioureido moiety (C-11), while the resonances at δ173.3, 170.5 and 171.9 ppm were assigned to the carbonyl groups of the

CO$_2$Meresidues, respectively. The carbonyl carbon atoms (C-9) appeared at δ171.0, 172.3 and 173.0 ppm, respectively, while the signals oriented at δ*δ* 155.5, 155.3 and 151.1 ppm were attributed to *C*-OMe carbon atoms. C-8 carbon atoms were resonated at δ29.9, 30.7 and 30.6 ppm, and C-13 at δ58.3, 60.4 and 69.6 ppm, respectively. The proton spin system of 31 was further identified from DFQ-COSY [37] spectrum, where the doublet of doublets of H_{valin}-13at δ_H 3.24 ppm was found to correlate with C-13 carbon atom at δ_C 60.4 ppm.

Next, indomethacin 1 was treated with various amino acid methyl esters (glycine, l-alanine, l-serine, l-valine, l-leucine and l-lysine acetates hydrochloride) in the presence of 1-hydroxybenzotriazole (HOBT) and dicyclohexylcarbodiimide (DCC) as coupling reagents to give 36-41 in 64% -79% yield (Scheme 3).

The structures of 36-41 were determined by their ^{1}H, and ^{13}C NMR, which showed a similar pattern of indole and aromatic protons. The low field doublets at the regions δ 8.63-8.38 ppm were assigned to NH group ($J_{NH,H11}$ ~ 5.5 Hz). CH_2-8 protons appeared as singlets at the regions δ 4.41-3.57 ppm, while H-11 protons of the amino acid residues (CONH*CH*) resonated as doublet or doublet of doublets at δ 4.50-3.78 ppm ($J_{NH,H11}$ ~ 5.5 Hz for doublet, and ~ 8.0 Hz, ~ 5.5 Hz for doublet of doublets). CH_2-11 of glycine residue of 36 appeared as doublet at δ 3.78 Hz (*J* = 2.2 Hz), while H-11 protons of 37-41 were resonated as doublet of doublets or mutiplet at the regions δ 4.50-4.18 (*J* ~ 7.9, 5.6 Hz). The other protons of aromatic, indole and amino acid moieties were fully analyzed (*cf*. Experimental section). In the ^{13}C NMR spectra of 37-41, the carbon atoms of the carboxylate groups resonated at the regions δ 173.2-172.4 ppm, while the carbonyl carbon atoms (C9=O) of the amide moiety resonated at the regions δ 171.6-170.0 ppm. The resonances at the regions δ 33.3-29.9 ppm were attributed to the carbon atoms of C-8, while the resonances at the regions δ 62.1-55.3 ppm were assigned to C-11 of the amino acid residues, except for 36 which appeared at δ 43.5 ppm. The CH*Me*, CH_2OH carbon signals of 37and 38 appeared at δ 18.2 and 26.2 ppm, respectively, while C-12 - C-15 of the l-lysine residue were resonated at δ 30.7, 24.4, 28.9 and 47.7 ppm, respectively. Compound 41 has been selected for further NMR study. Thus, a gradient selected HMBC spectrum [38] of 41 allowedthe identification of CH_2-8of the amide group at δ_H 3.54 ppm from the $^2J_{C,H}$ correlations to C-3a of the indole ring at δ_C 135.2 ppm and carbonyl carbon atom (C-9) of l-lysine residue at δ_C 170.0 ppm. Similarly, H-11 proton at δ_H 4.50 ppm was identified from it's a $^3J_{C,H}$ correlations to C-9 at δ_X 170.0 ppm and C-13 at δ_C 24.4 ppm, as well as two $^2J_{C,H}$ correlations: one with the carbonyl atom of the acetate group at δ_C 173.0 ppm and the other with C-12 at δ_C 30.7 ppm. C-14 at δ_C 28.9 ppm showed a $^2J_{C,H}$ correlation with CH_2-13 at δ_H 1.60 ppm, while 4-H of the indole ring at δ_H 6.96 ppm showed the same correlation with C-3a at δ_C 135.2 ppm. Additionally, The carbon atom (C-5) of the indole ring at δ_C 156.6 ppm showed a $^3J_{C,H}$ correlation with 7-H at δ_H 7.08 ppm (Fig. 2). Additionally, all the structures of the new synthesized indomethacin analogues have been identified by the ^{1}H, ^{13}C HSQC NMR spectroscopy [39].



Entry	R	Amino acid		R	Entry
36	H	glycine	L-valine	$CH(Me)_2$	**39**
37	Me	L-alanine	L-leucine	CH_2CHMe_2	**40**
38	CH_2OH	L-serine	L-lysine	$CH(CH_2)_4NH_2$	**41**

Scheme 3. Synthesis of alkyl 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-yl) acetamido carboxylate derivatives

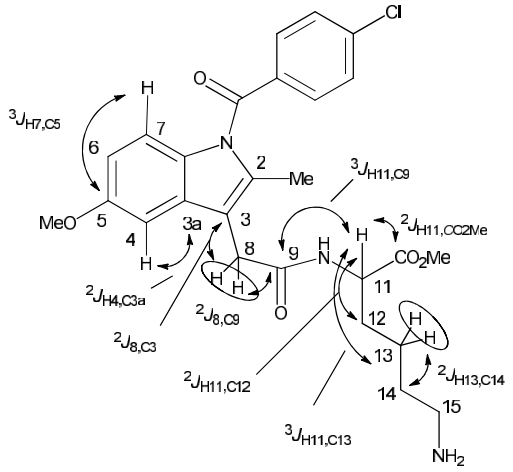


Fig. 2. $J_{C,H}$ correlations in the HMBC NMR spectrum of 41

3.2 Bioactivities

3.2.1*In-vitro* anti-HIV activity

Compounds 17-28, 29-30 and 33-41 were tested for their *In vitro* anti-HIV-1 (strain III_B) and HIV-2 (strain ROD) activity in human (MT-4) cells based on an MTT assay [28,29]. The results are summarized in Table 1, in which the data for nevirapine (BOE/BIRG587) [40]and azidothymidine (DDN/AZT) [41] were included for comparison purposes. Compound-induced cytotoxicity was also measured in MT-4 cells parallel with the antiviral activity.

Table 1. *In-vitro* anti-HIV-1[a] and HIV-2[b] activity and cytotoxicity of some new indomethacin analogues

Entry	HIV-1 (III_B) IC_{50} (µM)[c]	HIV-2 (ROD) IC_{50} (µM)[c]	CC_{50} (µM)[d]	SI[e] (III_B)	SI[e] (ROD)
4	>32.70	>32.70	32.70	<1	<1
17	>106.00	>106.00	≥106.00	<orX1	<orX1
18	>99.53	>99.53	99.53	<1	<1
19	> 90.90	>90.90	≥90.90	<orX1	<orX1
20	>100.00	>100.00	≥ 100.00	<orX1	<orX1
21	>86.70	>86.70	86.70	<1	<1
22	>104.00	>104.00	≥ 104.00	< orX1	< orX1
23	>67.25	>67.25	67.25	<1	<1
24	>92.68	>92.68	92.68	<1	<1
25	>77.88	>77.88	77.88	<1	<1
26	>103.75	>103.75	103.75	<1	<1
27	>125.00	>125.00	125.00	<1	<1
28	>101.13	>101.13	101.13	<1	<1
29	>12.33	>12.33	12.33	<1	<1
30	>8.83	>8.83	8.83	<1	<1
31	>1.81	>1.81	3.31	6	<1
33	>2.23	>2.23	2.23	<1	<1
34	>11.10	>11.10	11.10	<1	<1
35	>11.80	>11.80	11.80	<1	<1
36	>2.13	>2.13	2.13	<1	<1
37	>10.28	>10.28	10.28	<1	<1
38	>2.06	>2.06	2.06	<1	<1
39	>3.21	>28.89	28.89	9	<1
40	>7.27	>7.27	7.27	<1	<1
41	>1.98	>1.98	1.98	<1	<1
Nevirapine	0.050	>4.00	> 4.00	>80	<1
AZT	0.0022	0.00094	>25	>11363	>26596

[a]Anti-HIV-1 activity measured with strain III_B; [b] anti-HIV-2 activity measured with strain ROD; [c]compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 and HIV-2-induced cytopathic effect; [d]compound concentrationthat reduces the viability of mock-infected MT-4 cells by 50 %; [e] SI: selectivity index (CC_{50}/IC_{50})

None of these derivatives showed anti-HIV activity, except 31 and 39 with IC_{50} values of >1.81 and > 3.21 µM (CC_{50} of 3.31 and 28.89µM), resulting in selectivity index (SI) of 6 and 9, respectively. Derivatives 33, 36, 38 and 41 demonstatred lowCC_{50} values of >2.15, >3.03, >2.29, and >1.63 µM, respectively, at concentration of 100 µM, in comparison to the other analogues. However, the above data suggested that the arylnitril as a keto moiety (e.g.: 31) considerably increased the anti-HIV activity, in comparison to the effectiveness of other functional groups and to the other anti-HIV drug having nitrile residue such as rilpivirine [42].

3.2.2 *In-vitro* anti-HCV activity

Compounds 28, 31 and 32 were selected for evaluation of their *In vitro* selective anti-hepatitis C virus (HCV) activity in the Huh 5-2 replicon system (type 1b, Con1 strain) [33]. The compounds showed EC_{50} of 9.56, 8.27 and 10 µM with CC_{50} of 46.6, 27.8 and 28 *µ*M, resulting in a selectivity index (SI) of 4.88, 3.37 and 2.80, in addition to inhibition of 67.2,

69.7 and 69.3%, respectively. However, none of these compounds matched the selectioncriteria of a selective inhibitor of virus replication in this assay (i.e. >70% inhibition at concentrations that do not elicit an anti-metabolic effect on the host cells).

3.2.3 *In vitro* anti-Kinesin Eg5 activity

Antimitotic agents that have been used so far in cancer treatment, such as taxanes and vinca alkaloids, perturb tubulin polymerization/depolymerization, cause mitotic arrest and subsequent cell death [43]. However, these drugs produce serious side effects because microtubules also have essential intracellular functions in non-dividing cells [44]. A new alternative approach to prevent mitotic-spindle formation is the inhibition of proteins, such as the mitotic motors (kinesins) that interact with microtubules and cause mitotic arrest [45].

Monastrol (MA) has been reported as one of these antimitotic agents arrests cells in mitosis by specifically inhibiting Eg5 [46], a member of the Kinesin-5 family. Like many enzyme inhibitors, monastrol might be substrate competitive, inhibiting the ATP hydrolysis cycle of Eg5 by directly competing with ATP [45,47,48], or microtubule binding. Alternatively, monastrol might inhibit the motor domain allosterically, either by inhibiting ATP hydrolysis or by uncoupling partner head interactions to inhibit motor but not ATPase activity [49,50].

Compounds 17-28 have been selected for their inhibition of Eg5 activity using an *In vitro* malachite green ATPase assay (enzyme-coupled assay) [51]. None of these compounds matched the selection criteria of a selective inhibitor of Eg5 in this assay in comparison to monastrol, except 27 which showed ATPase inhibition value of 48% at 100 µMconcentration and inhibition of kinesin Eg5 with IC_{50} of 4.87 µM. From the data, it appears that a trifluoromethyl group on the 4-position of aromatic ring is important for Eg5 inhibition.

4. CONCLUSION

In this paper we have reported the synthesis and anti-HIV-1 and anti-HIV-2 evaluation of some indomethacin derivatives having bi- and triaryl groups as well as thioureido and amido residues of some l-amino acids. The preliminary *In vitro* anti-HIV activity of these analogues are too limited to perform extensive mode-of-action studies, however, 31 and 36 might be a new lead in the development of antiviral agents as nonnucleoside reverse transcpriptase inhibitors (NNRTIs). In addition, we reported the *In vitro* anti-HCV activity of three analogues but did not showed a selection inhibition of virus replication. Our study included the inhibition study of kinesin Eg5 *via* the ATPase activity assay, and the result indicated that 27, the only analouge of the indomethacin series showed moderate inhibition of ATPase activity (48%) at concentration of 100 µM.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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