Original Article

Design and Synthesis of Novel Indolizine Analogues as COX-2 Inhibitors: Computational Perspective and *in vitro* Screening

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ABSTRACT

Design and synthesis of a new series of ethyl 7-methoxy-2-substituted-3-(substituted benzoyl) indolizine-1-carboxylates **2a-i** was achieved and screened for their *in vitro* inhibitory activity against COX-2 enzyme. Compound **2a** and **2c** emerged as promising COX-2 enzyme inhibitor with IC_{50} of 6.56 and 6.94 μM respectively from the synthesized series when compared to Celecoxib and Indomethacin as selective and nonselective standards, respectively. Computational docking study identified the possible reasons for such activity that may be due to the *cis* configuration of the indolizines that resulted in the most stable conformation similar to that of Indomethacin.

Key words: Cox-2 Inhibition, Indolizine Analogues, Synthesis, Characterization, Molecular Docking.

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INTRODUCTION

Indolizines are bicyclic heterocyclic compounds with various promising pharmacological properties.[1-3] With regard to positions there are nine non-equivalents around the bicyclic indolizine structure. Several synthetic strategies have been adopted to obtain substituted indolizine analogues with various functional groups.[1, 4-7] Some of the biologically important natural products and synthetic pharmaceuticals contain indolizine pharmacophore as an important *N*-fused heterocycles.[8-18] Accordingly, synthesis and derivatization of indolizines have attracted considerable attention of medicinal chemist over the decades.[19-33] Particularly the 3-benzoylindolizines are attractive since their derivatives have been used as pharmacologically interesting compounds and their vital role as the synthetic intermediates for 3-substituted indolizines is also apparent.[34] Indolizine system is isoelectronic with indole nucleus and signifies a group of heterocyclic compounds structurally associated to purines. Several indolizine analogues have been reported for various pharmacological properties such as analgesic,[35,36] anti-inflammatory,[37] 5HT3 receptor antagonist,[38] anticholinergic,[39] anticancer,[40-42] estrogen receptor binding,[43] antioxidant,[44,45] antimicrobial,[46] antimutagenic,[47] CNS depressant[48] and hypoglycemic activities.[49,50] Cyclooxygenase (COX) enzyme mainly occurs in two isoforms COX-1 and COX-2 that have 60% identity of their sequence and the latter one is the key enzyme

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in the biosynthetic pathway leading to the development of prostaglandins, which are mediators of inflammation.[51] The conventional nonsteroidal anti-inflammatory drugs (NSAIDs) tend to exhibit unwanted side effects such as gastrointestinal, cardiovascular and renal complications.[52] Literature survey reveals that the overexpression of particularly cyclooxygenases-2 enzyme, promotes multiple events involved in tumorigenesis; in addition, several studies demonstrates that the inhibition of cyclooxygenases-2 can delay or prevent certain forms of cancer.[53]

In continuation of our studies on polymorphism behavior of heterocyclic compounds[54-56] and pharmacological screening of heterocyclic compounds for anticancer,[40,57] anti-mosquito[58,59] and anti-TB[60] properties, herewith we undertake design of proposed compounds based on Lipinski rule of five[61] and synthesis of novel ethyl 3-(substitutedbenzoyl)-7-methoxy-2-methylindolizine-1-carboxylates (Scheme-1) and screen them for *in vitro* COX-2 inhibitory activity. In addition, computational studies to study the conformational impact on the binding and inhibitory activity.

MATERIALS AND METHODS

General chemistry

All the commercially available chemicals were purchased from Sigma-Aldrich chemicals and all the chemical reactions were carried out in hot-air dried glass wares under nitrogen atmosphere using dry solvents. NMR (300, 400 MHz) spectra were recorded at ambient temperature using $CDCl_3$ and DMSO-d_6 as a solvent using Bruker-400 spectrometer. Chemical shift values are measured in δ ppm and were referenced with tetramethylsilane (TMS). The peak multiplicities were given as *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet; *m*, multiplet. LC-MS analysis was performed on Agilent LC-1200 series coupled with 6140 single quad mass spectrometer with ESI +ve and –ve mode, MS range 100-1500. Perkin Elmer CHNS analyser was used to perform elemental analysis.

GENERAL PROCEDURE FOR THE SYNTHESIS OF 4-METHOXY-1-(2-(SUBSTITUTEDPHENYL)-2-OXOETHYL)PYRIDINIUM BROMIDE (1A-D)

TO A STIRRED solution of 4-methoxypyridine (0.0091 mol) in dry acetone (10 mL), 4-substituedphenacylbromide (0.0091 mol) was added and at room temperature the reaction mixture was stirred for 5 h. Completion of the reaction was checked on thin layer chromatography. The product separated was filtered, recrystallized using ethanol as solvent and dried at room temperature to afford 98-99% yield of 1-(2-(4-substituedphenyl)-2-oxoethyl)-4-methoxypyridinium bromides **1a-d**.

1-(2-(4-Cyanophenyl)-2-oxoethyl)-4-methoxypyridinium bromide (1a)

Appearance: Light yellow colour solid. Yield 98%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ = 8.96-8.94 (d, J = 7.2 Hz, 2H), 8.52-8.50 (d, J = 7.0 Hz, 2H), 7.90-7.88 (d, J = 7.2 Hz, 2H), 7.58-7.56 (d, J = 8 Hz, 2H), 6.29 (s, 2H), 4.14 (s, 3H); LC-MS (ESI, Positive): m/z: (M+H)$^+$: 253.2.

1-(2-(4-Fluorophenyl)-2-oxoethyl)-4-methoxypyridinium bromide (1b)

Appearance: White colour solid. Yield 99%. ^{1}H-NMR (400 MHz , DMSO-d_6) δ = 8.72-8.70 (d, J = 7.0 Hz, 2H), 8.75-8.67 (d, J = 7.2 Hz, 2H), 8.30-8.27 (m, 2H), 8.13-8.10 (t, J = 8.8 Hz, 2H), 6.28 (s, 2H), 4.19 (s, 3H); LC-MS (ESI, Positive): m/z: (M+H)$^+$: 246.12.

1-(2-(4-Bromophenyl)-2-oxoethyl)-4-methoxypyridinium bromide (1c)

Appearance: White colour solid. Yield 98%. ^{1}H NMR (400 MHz , DMSO-d_6) δ = 9.10-9.08 (d, J = 7.2 Hz, 2H), 8.24-8.22 (d, J = 7.0 Hz, 2H), 8.02-8.00 (d, J = 7.2 Hz, 2H), 7.72-7.70 (d, J = 8 Hz, 2H), 6.24 (s, 2H), 4.15 (s, 3H); LC-MS (ESI, Positive): m/z: (M+H)$^+$: 306.2.

4-Methoxy-1-(2-(3-methoxyphenyl)-2-oxoethyl) pyridinium bromide (1d)

Appearance: Yellow colour solid. Yield 99%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ = 9.02-9.00 (d, J = 7.2 Hz, 2H), 8.54-8.52 (d, J = 7.2 Hz, 2H), 7.49-7.47 (d, J = 7.2 Hz, 1H), 7.36-7.31 (t, J = 7.2 Hz, 1H), 7.15 (s, 1H), 7.10-7.08 (d, J = 7.2 Hz, 1H), 6.27 (s, 2H), 4.10 (s, 3H), 3.88 (s, 3H); LC-MS (ESI, Positive): m/z: (M+H)$^+$: 258.2.

General procedure for the synthesis of ethyl 7-acetyl-3-(substitutredbenzoyl)-2-substitutedindolizine-1-carboxylate (2a-i)

To a stirred solution of 1-(2-(substitutedphenyl)-2-oxoethyl)-4-methoxypyridinium bromide (0.00156 mol), in dry dimethylformamide (15mL), was added ethyl propiolate (0.00156 mol) and K_2CO_3 (0.0031 mol). At room temperature the reaction mixture was stirred for 30 min and reaction completion was monitored on TLC. After reaction completion, the solvent was evaporated under reduced pressure and diluted with ethyl acetate. Water and brine was used to wash organic layer and dried over sodium sulphate. The crude compound was purified by column chromatography to afford 66-80 % yield of compounds **2a-i**. Physicochemical constants of the characterized compounds are tabulated in Table 1.

COX-2 inhibition activity

The title compounds **2a-i** were screened for human recombinant COX-2 inhibitory activity using an enzyme immunoassay kit. IC_{50} is the concentration of test and standard compounds required to produce 50% inhibition of human recombinant COX-2 by means of three determinations using the enzyme linked immuno sorbent assay kit (Table 2). Significant differences were detected between treatments ($F_{10,32}$ = 108.7; p< 0.001). Test compound **2a** with nitrile group on phenyl ring which is connected to indolizine nucleus through carbonyl group emerged as promising COX-2 enzyme inhibitor with IC_{50} of 6.56 μM from the series when compared to selective (Celecoxib) and nonselective (Indomethacin) standard compounds with their inhibitory activity at 0.05 and 6.8 μM, respectively. Compound **2c** exhibited moderate COX-2 enzyme inhibition activity with IC_{50} of 6.94 μM.

Computational Studies

Molecular docking

The crystal structures of COX-1 and COX-2 have sequence identity of 60 % [68] The active site of both isoforms have a typical active site that have a hydrophobic long channel. Mapping of this channel illustrated that it has a number of hydrophobic residues such as Leu 384, Phe 381, Tyr 385, and Trp 387 beside other residues such as Arg 120, His 90. Non-selective NSAIDs are all bound to this hydrophobic channel by interactions of their carboxylate anionic group with the cationic guanidinium group of Arg 120 forming salt bridge. In addition, they form hydrogen bond with Tyr 355.

The binding of Indomethacin as a non-selective COX inhibitor has shared binding mode in both COX-1 and COX-2 hydrophobic channel in which the chloro atom at *para*-phenyl moiety interacts with Leu 384. The benzoyl carbonyl C=O group is essential in hydrogen bond formation with the hydroxyl group of Ser 530 and Val 349. The phenyl part of the benzoyl moiety shared by hydrophobic interactions with Leu 384, Phe 381, Tyr 385, and Trp 387. The indole scaffold participates by a hydrophobic interactions as well with Val 349.[68,69]

Indomethacin has many conformations but, the *cis* conformation around the C=O in which both *para*-chloro phenyl moiety and indole moiety are oriented in the same direction has achieved higher selectivity toward COX-2 inhibition [70].

The main difference between COX-1 and COX-2 binding site is the presence of hydrophilic side pocket in COX-2 only just beyond the hydrophobic channel. The selective COX-2 inhibitor Celecoxib has a hydrophilic sulfonyl amino side chain (H_2N-SO_2) that allows the drug to fit in this hydrophilic side pocket and forming strong hydrogen bonding with His 90, Gln 192 and Arg 513. Celecoxib does not have a carboxylic group as well so, the salt bridge with Arg 120 is not found. In other words, the binding mode of non-selective COX inhibitors is different from those of selective ones due to structural basis in COX-2 isoform itself. Also. Celecoxib does not inhibit COX-1 at therapeutic concentrations effective on COX-2.

In this work, a series of novel indolizine analogues has been synthesized and tested for the COX-2 inhibition. A molecular docking of the synthesized compounds against both COX-1 and COX-2 was done to interpret their possible binding mode. According to the docking results (Table 3), the docked compounds almost have the same in silico affinity of Indomethacin. All

Table 1: Physicochemical constants of ethyl 7-methoxy-3-(substitutredbenzoyl)-2-substitutedindolizine-1-carboxylate analogues 2a-i.

Compound	Mol formulae (Mol mass)	R^1	R^2	Yield (%)[a,b]	m.p (°C)	*c*LogP[c]
2a	$C_{20}H_{16}N_2O_4$ (348)	4-CN	H	76	165	3.9570
2b	$C_{21}H_{18}N_2O_4$ (362)	4-CN	CH_3	68	191	4.4560
2c	$C_{19}H_{16}FNO_4$ (341)	4-F	H	79	118	4.5293
2d	$C_{20}H_{18}FNO_4$ (355)	4-F	CH_3	72	137	5.0283
2e	$C_{21}H_{20}FNO_4$ (369)	4-F	C_2H_5	70	124	5.5573
2f	$C_{19}H_{16}BrNO_4$ (402)	4-Br	H	74	183	5.2493
2g	$C_{20}H_{18}BrNO_4$ (416)	4-Br	CH_3	70	148	5.7483
2h	$C_{20}H_{19}NO_5$ (353)	3-OCH_3	H	80	116	4.4986
2i	$C_{21}H_{21}NO_5$ (367)	3-OCH_3	CH_3	74	130	4.9976

[a] Compounds **2a-i** were characterized by physical and spectral data.
[b] Yields after purification by column chromatography.
[c] *c*Log*P* was calculated using ChemBioDraw Ultra 13.0v.

Table 2: *In vitro* COX-2 inhibitory activity of ethyl 7-methoxy-3-(substitutredbenzoyl)-2-substitutedindolizine-1-carboxylate analogues 2a-i.

Compound	Substituents		IC_{50} (μM) [a]
	R^1	R^2	
2a	4-CN	H	6.56±0.03[ab]
2b	4-CN	CH_3	7.24±0.03[c]
2c	4-F	H	6.94±0.03[cd]
2d	4-F	CH_3	7.52±0.03[e]
2e	4-F	C_2H_5	7.95±0.03[e]
2f	4-Br	H	7.27±0.03[c]
2g	4-Br	CH_3	7.54±0.03[e]
2h	3-OCH_3	H	7.36±0.03[f]
2i	3-OCH_3	CH_3	7.35±0.03[f]
Indomethacin	-	-	6.84±0.03[bd]
Celecoxib	-	-	0.05±0.03[a]

[a] IC_{50} value is the concentration of test and standard compounds required to produce 50% inhibition of human recombinant COX-2 by means of three determinations using the enzyme linked immuno sorbent assay kit. IC_{50} value not sharing the same superscript letter differ significantly (p<0.05).

Table 3: Molecular docking results of ethyl 7-methoxy-3-(substitutredbenzoyl)-2-substitutedindolizine-1-carboxylate analogues 2a-i.

Compound	Computational binding affinity (Kcal/mol)	
	COX-1	COX-2
2a	-6.95	-7.51
2b	-6.50	-7.15
2c	-6.75	-7.35
2d	-6.45	-7.20
2e	-6.60	-7.30
2f	-6.35	-6.95
2g	-6.30	-6.90
2h	-6.25	-6.75
2i	-6.27	-6.70
Indomethacin	-7.05	-7.65
Celecoxib	-11.32	-13.85

compounds shared a common binding mode in the hydrophobic channel of COX-2 in which the substituted benzoyl moiety and the indolizine ring were oriented to the same direction. The best poses for the active compounds **2a** and **2c** have been superimposed on the best pose of Indomethacin as well (Figure 1).

Ethyl 3-(4-cyanobenzoyl)-7-methoxyindolizine-1-carboxylate (2a)

Appearance: Yellow colour fluffy crystalline compound; IR (neat cm^{-1}): 2227, 1701, 1639, 1604; ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 9.82-9.79 (d, *J* = 7.2 Hz, 1H), 7.77 (s, 1H), 7.76-7.64 (m, 5H), 6.81-6.77 (m, 1H), 4.41-4.33 (q, *J* = 7.2Hz, 2H), 3.99 (s, 3H), 1.43-1.38 (t, *J* = 7.2 Hz, 3H). LC-MS (ESI, Positive): *m/z*: $(M+H)^+$ = 349.2; Anal. calculated for $C_{20}H_{16}N_2O_4$: C, 68.96; H, 4.63; N, 8.04; Found : C, 68.82: H, 4.61: N, 8.07.

Ethyl 3-(4-cyanobenzoyl)-7-methoxy-2-methylindolizine-1-carboxylate (2b)

Appearance: Light yellow colour crystalline compound; IR (neat cm^{-1}): 2231, 1689, 1631, 1591; ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 9.58-9.56 (d, *J* = 7.6 Hz, 1H), 7.79 (s, 1H), 7.78-7.76 (d, *J* = 6.4 Hz 4H), 7.72-7.70 (d, *J* = 6.4 Hz, 1H), 6.71-6.69 (m, 1H), 4.39-4.34 (q, *J* = 7.2 Hz, 2H) , 3.95 (s, 3H), 2.11 (s, 3H), 1.42-1.39 (t, *J* = 7.2Hz, 3H); LC-MS (ESI, Positive): *m/z*: $(M+H)^+$ = 363.12; Anal. calculated for $C_{21}H_{18}N_2O_4$: C, 69.60; H, 5.07; N, 7.73; Found : C, 69.59: H, 4.98: N, 7.79.

Ethyl 3-(4-fluorobenzoyl)-7-methoxyindolizine-1-carboxylate (2c)

Appearance: Light brown colour crystalline compound; IR (neat cm^{-1}): 1695, 1641, 1614; ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 9.79-9.77 (d, *J* = 7.5 Hz, 1H), 7.85-7.80 (m, 2H), 7.73 (s, 1H), 7.68 (s, 1H), 7.26-7.15 (m, 2H), 7.25-7.21 (t, *J* = 8.4Hz, 2H), 6.78-6.75 (m, 1H), 4.37-4.33 (q, *J* = 7.2 Hz, 2H), 3.97 (s, 3H), 1.41-1.36 (t, *J* = 7.2 Hz, 3H); LC-MS (ESI, Positive): *m/z*: $(M+H)^+$ 342.2; Anal. calculated for $C_{19}H_{16}FNO_4$: C, 66.86; H, 4.72; N, 4.10; Found : C, 66.82: H, 4.81: N, 3.99.

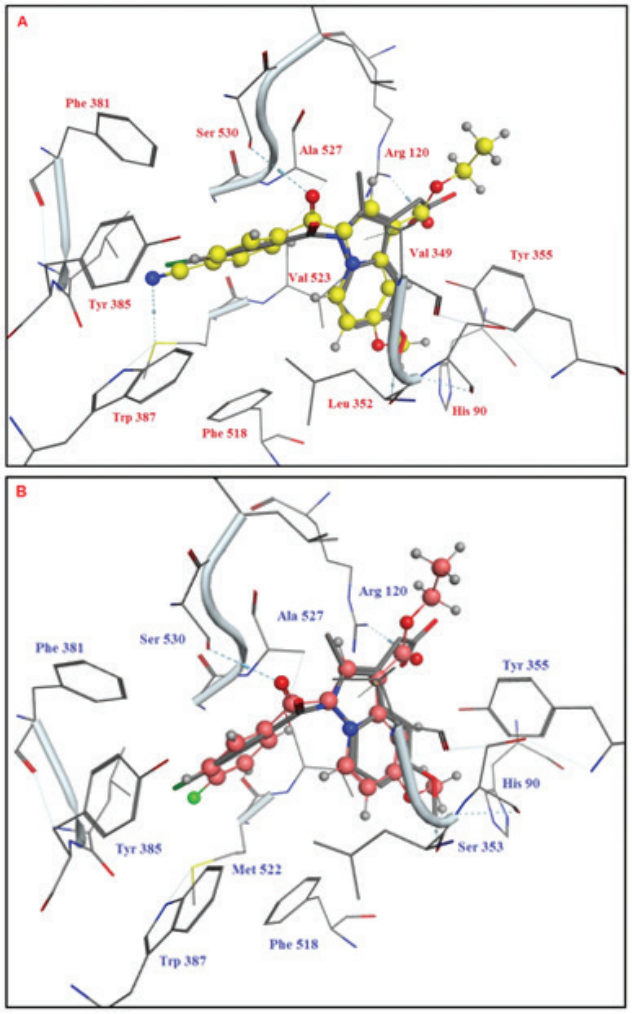


Figure 1: A) Binding mode of compound 2a in yellow colour superimposed with Indomethacin (elemental). B) Binding mode of compound 2c in pink colour superimposed with Indomethacin (elemental).

The analysis of the best poses of compounds 2a and 2c it was obvious that the compounds showed hydrogen bond formed by its benzoyl C=O group which was close to Ser 530 hydroxyl group than that of Indomethacin. The carboxylate C=O formed another hydrogen bond with Arg 120 with distance 2.98 A° when compared with that of Indomethacin 3.04 A°. The present of nitrile group at the para position of 2a allows dipole interactions with Met 522. The synthesized compounds do not have any hydrophilic side chains like Celecoxib and when Celecoxib was docked in COX-2 it showed different interaction pattern (Figure 2)

Ethyl 3-(4-fluorobenzoyl)-7-methoxy-2-methylindolizine-1-carboxylate (2d)

Appearance: Light brown colour crystalline compound; IR (neat cm^{-1}): 1672, 1641, 1602; ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 9.41-9.39 (d, *J* = 7.8 Hz, 1H), 7.76-7.68 (m, 3H), 7.17-7.14 (m, 2H), 6.68-6.64 (m, 1H), 4.42-4.35 (q, *J* = 7.2 Hz, 2H), 3.96 (s, 3H) , 2.19 (s, 3H), 1.45-1.40 (t, *J* = 7.2 Hz, 3H); LC-MS (ESI, Positive): *m/z*: $(M+H)^+$ 356.2; Anal. calculated for $C_{20}H_{18}FNO_4$; C, 67.60; H, 5.11; N, 3.94; Found; C, 67.56; H, 5.16; N, 3.88.

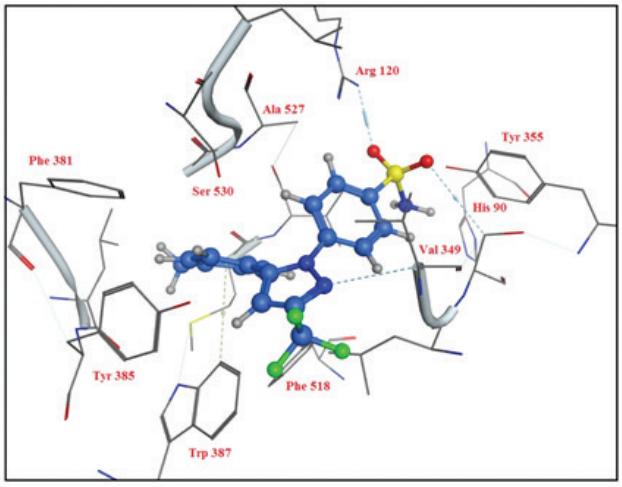


Figure 2: Binding mode of Celecoxib in COX-2 binding site.

It showed hydrogen bond between Arg 120 and the sulfonyl oxygen. The second oxygen atom of sulfonyl group showed a hydrogen bond with His 90. According to the previous docking results, it was clear that the structure similarity with Indomethacin allows the indolizine to share the same binding mode. However, they have different structural features like; the absence of methylene group attached to carboxylic moiety in Indomethacin and that was a feature in making their C=O group close to Arg 120.

The position of nitrogen atom in indole scaffold is different than that of indolizine which allow the conformation of the indolizine to be in its stable form in the cis conformation (Figure 3).

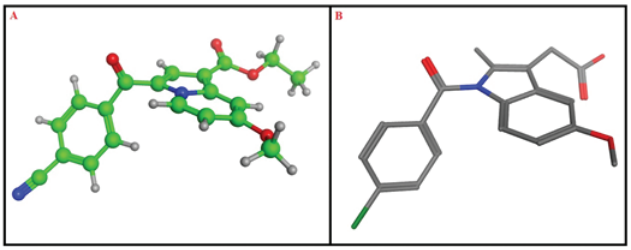


Figure 3: Comparison of the stable conformations of both A) indolizine active analogue 2a and B) Indomethacin.

Ethyl 2-ethyl-3-(4-fluorobenzoyl)-7-methoxyindolizine-1-carboxylate (2e)

Appearance: Light yellow colour crystalline compound; IR (neat cm^{-1}): 1668, 1641, 1591; ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 9.25-9.23 (d, *J* = 7.6 Hz, 1H), 7.76 (s, 1H), 7.70-7.67 (m, 2H), 7.17-7.12 (m, 2H), 6.64-6.61 (m, 1H), 4.40-4.35 (q, *J* = 7.2 Hz, 2H), 3.94 (s, 3H), 2.68-2.62 (q, *J* = 7.2 Hz, 2H), 1.43-1.39 (t, *J* = 7.2Hz, 3H), 1.00-0.97 (t, *J* = 7.2Hz, 3H); LC-MS (ESI, Positive): *m/z* $(M+H)^+$ = 370.12; Anal. calculated for $C_{21}H_{20}FNO_4$: C, 68.28; H, 5.46; N, 3.79; Found: C, 68.29: H, 5.47: N, 3.71.

Ethyl 3-(4-bromobenzoyl)-7-methoxyindolizine-1-carboxylate (2f)

Appearance: Light brown colour crystalline compound; IR (neat cm^{-1}): 1699, 1647, 1606; ^{1}H-NMR (300 MHz,

$CDCl_3$) δ = 9.82-9.79 (d, *J* = 7.2 Hz, 1H), 7.89-7.86 (m, 2H), 7.83-7.75 (m, 4H), 7.62 (s, 1H), 6.82-6.78 (m, 1H), 4.39-4.34 (q, *J* = 7.2 Hz, 2H), 3.98 (s, 3H), 1.41-1.35 (t, *J* = 7.2Hz, 3H); LC-MS (ESI, Positive): *m/z*: $(M+H)^+$ 402.2, 404.4; Anal. calculated for $C_{19}H_{16}BrNO_4$: C, 56.73; H, 4.01; N, 3.48; Found : C, 56.72: H, 3.97: N, 3.49.

Ethyl 3-(4-bromobenzoyl)-7-methoxy-2-methylindolizine-1-carboxylate (2g)

Appearance: Light brown colour crystalline compound; IR (neat cm^{-1}): 1697, 1670, 1639; ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 9.44-9.41 (d, *J* = 7.5 Hz, 1H), 7.74-7.51 (m, 5H), 6.67-6.64 (m, 1H), 4.40-4.33 (q, *J* = 7.2 Hz, 2H) , 3.94 (s, 3H), 2.17 (s, 3H), 1.43-1.38 (t, *J* = 7.2 Hz, 3H); LC-MS (ESI, Positive): *m/z* $(M+H)^+$ = 416.2, 418.2; Anal. calculated for $C_{20}H_{18}BrNO_4$: C, 57.71; H, 4.36; N, 3.36; Found : C, 57.76; H, 4.31; N, 3.37.

Ethyl 7-methoxy-3-(3-methoxybenzoyl)indolizine-1-carboxylate (2h)

Appearance: Light brown colour crystalline compound; IR (neat cm^{-1}): 1696, 1672, 1645, 1608; ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 9.82-9.79 (d, *J* = 7.2 Hz, 1H), 7.74 (s, 1H), 7.44-7.31 (m, 4H), 7.12-7.08 (m, 1H), 6.78-6.74 (m, 1H), 4.38-4.33 (q, *J* = 7.2 Hz, 2H) 3.96 (s, 3H), 3.87 (s, 3H) 1.40-1.35 (t, *J* = 7.2 Hz, 3H); LC-MS (ESI, Positive): *m/z* $(M+H)^+$ = 354.2; Anal. calculated for $C_{20}H_{19}NO_5$; C, 67.98; H, 5.42; N, 3.96; Found; C, 67.95; H, 5.44; N, 3.93.

Ethyl 7-methoxy-3-(3-methoxybenzoyl)-2-methylindolizine-1-carboxylate (2i)

Appearance: Light brown colour crystalline compound; IR (neat cm^{-1}): 1701, 1666, 1641, 1598; ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 9.47-9.44 (d, *J* = 7.8 Hz, 1H), 7.76 (s, 1H), 7.41-7.38 (m, 1H), 7.38-7.24 (m, 2H), 7.11-7.08 (m, 1H), 6.68-6.65 (m, 1H), 4.42-4.37 (q, *J* = 7.2 Hz, 2H), 3.96 (s, 3H), 3.87 (s, 3H), 2.20 (s, 3H), 1.45-1.40 (t, *J* = 7.2Hz, 3H); LC-MS (ESI, Positive): *m/z* $(M+H)^+$ = 368.2; Anal. calculated for $C_{21}H_{21}NO_5$; C, 68.56; H, 5.76; N, 3.81; Found; C, 68.54; H, 5.78; N, 3.79.

In vitro COX-2 inhibition activity

The synthesized test compounds **2a-i** were subjected for *in vitro* human recombinant COX-2 enzyme inhibitory activity using an enzyme immunoassay (EIA) kit according to a reported literature.[62-64]

Molecular docking with MOE 20.13.08

Molecular Operating Environment (MOE) 2013.08 package license was purchased from Chemical Computing Group Inc, Sherbooke St, Montreal, QC, Canada,[65] All the test compounds were built and saved as .MOE files. Rigid receptor was used as a docking protocol. Both receptor-solvent were kept as a "receptor". Triangle matcher was used as a placement method. Two rescoring were computed, rescoring 1 was selected as London dG. Rescoring 2 was selected as affinity. Force field was used as a refinement.

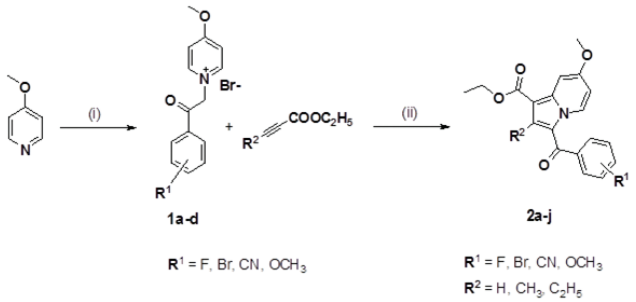
Statistical analysis

Comparison of *In vitro* COX-2 inhibitory activity of indolizine analogs with selective (Celecoxib) and non-selective (Indomethacin) standard compounds were carried out using one-way investigation of variance (ANOVA) that examined the main effect of treatment (ethyl 7-methoxy-3-(substitutredbenzoyl)-2-substitute-dindolizine-1-carboxylate analogues **2a-i,** Celecoxib and Indomethacin) on values of IC_{50}. Bonferroni test was used for post hoc analysis to account for the increased possibility of type I error.[66] Before ANOVA testing, data were transformed to ranks[67] to fit better the assumptions of the test. In all cases, a value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Scheme-1 describes the general route to obtain the title compounds **2a-i**. Intermediate compounds **1a-d** were prepared by stirring 4-methoxylpyridine with substituted phenacyl bromides separately in the presence of acetone medium at room temperature as shown in step-I of Scheme-1. The reaction completion was monitored on Thin Layer Chromatography (TLC) and after completion of the reaction, the solid deposited was filtered, dried at room temperature and recrystallized using ethanol as solvent. NMR and LC-MS method was used to characterize the compounds **1a-d** and yields obtained were in the range of 98-99%.

Substituted indolizine analogues **2a-i** have been prepared by the reaction between 4-methoxy-1-(2-(substituted phenyl)-2-oxoethyl)pyridin-1-ium bromide and substituted alkynes in dimethylformamide medium in presence of anhydrous potassium carbonate as depicted in step-II of Scheme-1. The completion of the reaction was monitored on TLC and all the products have been achieved within 30 min with constant stirring. The products were purified by column chromatography using 60-120 mesh silica gel using *n*-hexane - ethyl acetate as a solvent and the yield was found to be 68-80%. IR, NMR, LC-MS and elemental analysis methods were used to characterize the compounds **2a-i**. ChemBioDraw Ultra 13.0v was used to calculate *c*Log*P* of the compounds and the values were in the range of 3.9570-5.7483.



Scheme 1: Reagents and conditions: (i) substituted phenacyl bromide, acetone, 5 h, stir at room temperature; (ii) K_2CO_3, DMF, stir at room temperature, 30 min.

CONCLUSION

The reactions performed to obtain indolizine analogues **2a-i** are eco-friendly as they are carried out at room temperature with satisfactory yield. The compounds **2a** and **2c** emerged as promising compounds for COX-2 inhibition from the series when compared to standard substances Indomethacin and Celecoxib, which is also authenticated with docking studies.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found as attachment.

COMPETING INTERESTS

The authors declare that they have no competing interests.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ABBREVIATION USED

COX: Cyclooxygenase enzyme; **NMR:** Nuclear magnetic resonance; **TLC:** Thin layer chromatography; **LC-MS:** Liquid chromatography–mass spectrometry.

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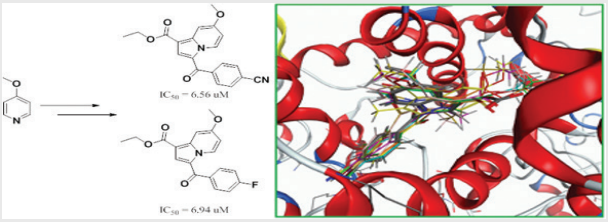
PICTORIAL ABSTRACT



SUMMARY

- Design and synthesis of a new series of ethyl 7-methoxy-2-substituted-3-(substitutedbenzoyl) indolizine-1-carboxylates **2a-i** was achieved by two step chemical reactions and screened for their *in-vitro* inhibitory activity against COX-2 enzyme.
- Compound **2a** and **2c** emerged as promising COX-2 enzyme inhibitor with IC_{50} of 6.56 and 6.94 μM, respectively from the synthesized series when compared to Celecoxib and Indomethacin as selective and nonselective standards, respectively.

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