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Biological evaluation and in silico molecular docking study of a new series of thiazol-2-yl-hydrazone conglomerates

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Abstract A new series of hybridized thiazol-2-yl-hydrazone derivatives having diverse substituents were designed, synthesized, and screened for their anti-inflammatory property by a carrageenan-induced paw edema method. The compounds **11a, 11b, 11c, 11d, 11e, 11g, 11m** and **11p** revealed significant inhibition when compared to Diclofenac sodium. Subsequently, two highly potent compounds (**11d** and **11e**) were evaluated for their cytotoxic effect on the tumor cell line. The binding interactions of thiazol-2-yl-hydrazones with the cyclooxygenase-2 (COX-2) protein (PDB: 3LN1) displayed effective interactions with Arg-120, Tyr-385 and Tyr-355 amino acids, the main criteria of the COX-2 inhibitor. In addition, all the compounds showed moderate to good in vitro antibacterial activity. Most active benzyloxy derivatives were also tested to understand the radical scavenging efficacy by the 2,2-diphenyl-1-picrylhydrazyl method.

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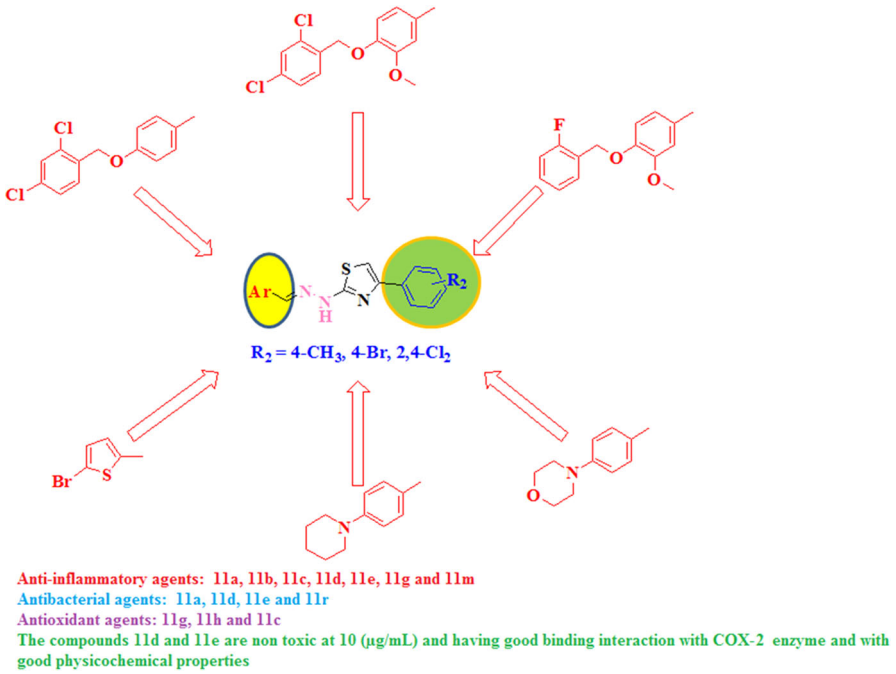
Graphical Abstract



Keywords Benzyloxy · Paw edema · Anti-inflammatory · Antibacterial · Anti-oxidant · COX-2

Introduction

In recent years, heterocycles have fascinated researchers as a vital scaffold of natural products. Of note, incorporation of small heterocyclic moieties especially a five-membered sulfur- and nitrogen-containing thiazole ring is an enthralling area of research in enhancing the potency of the drug candidate [1]. The literature has promulgated that 2,4-disubstituted thiazoles have prevalent biomedicinal utility and are also effective as anti-inflammatory [2–5], anticandida [6], antimicrobial, antibacterial, antibiotic, antimycobacterial, anti-oxidant, anticancer, antitumor, antiviral [7], and anti-Alzheimer [8] agents. On the other hand, thiazole acts as a potent inhibitor of H^+/K^+-ATPase [28], MAO-B [9], histone acetyltransferase [10], procaspase-3 kinase [11], and *Trypanosoma cruzi* [12] (Fig. 1).

Moreover, the benzyloxy group paves the way to develop novel molecules with their germaneness in the field of medicinal chemistry as an important pharmacophore. Pharmacological acceptance of the benzyloxy group has been materialized as a potential anticancer and anti-oxidant [13], anti-breast cancer [14],

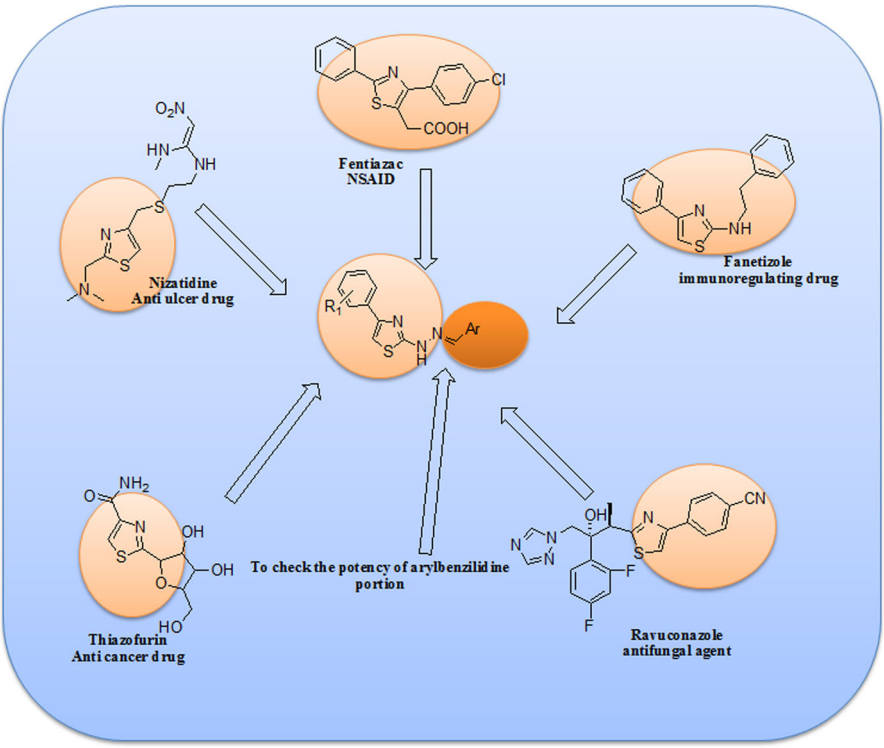


Fig. 1 Commercially available drugs which are incorporated with 2,4-disubstituted thiazoles and synthetic strategy of the series

antileishmanial [15], antiproliferative [16], antiprotozoal [17], antitubercular [18], and anticonvulsant [19] agents. They also act as inhibitors of *Plasmodium falciparum* [20] and monoamine oxidase B [21]. Additionally, morpholine and piperidine derivatives are known to possess varied medicinal applications [22–28].

In recent decades, the treatment of inflammatory disorders has become one of the major challenges for the scientists. However, non-steroidal anti-inflammatory drugs (NSAIDs) are the paramount therapeutics for these and act by inhibiting the COXs enzymes which are responsible for the prostaglandin biosynthesis. To date, three isoforms of COX enzymes have been identified as COX-1, COX-2 and COX-3 [29]. Interestingly, COX-2 is the responsible isoenzyme for the formation of prostaglandin from arachidonic acid which has been the chief reason of inflammation. On the other hand, COX-1 is mainly involved in maintaining the physiological action of the tissues [30]. Hence, COX-2 has been recognised as the appropriate target for the discovery of anti-inflammatory drugs. Moreover, NSAIDs existing from the past few decades have major side effects due to the lack of inhibitory specificity for the COX-2 enzyme [31]. Thus, there is a need for the development of the new NSAIDs having specificity with minimum toxicity.

Nevertheless, multi-drug resistance acquired by the microbes for the drugs, which are being extensively used for the microbial infection, creates an incessant

demand for the development of new drugs with high efficacy and less toxicity [32]. Additionally, the usage of multi-drugs for the treatment of an inflammatory disorder, concomitant with the microbial infection, may cause severe side effects in the patients with liver and kidney disorders [33]. The utility of monotherapy along with the multi-targeted drug is a major endeavor in curing inflammatory conditions and with advantages over multi-drug therapy [34, 35].

Furthermore, the role of anti-oxidants in alleviating the inflammation by scavenging the free radicals is never neglected and numerous anti-inflammatory agents have been reported to act by the same mechanism [36]. Hence, the active benzyloxy derivatives, which showed good anti-inflammatory activity, were evaluated to check their potency as radical scavengers.

All the aforementioned literature encouraged us to design and synthesize a series of simple, bioactive novel hybrids with diverse thiazol-2-yl hydrazones. Moreover, anti-inflammatory activity of various benzyloxy hydrazones was compared with the potency of (5-bromothiophen-2-yl)methylene), (4-(piperidin-1-yl)benzylidene), and (4-morpholino benzylidene) moieties. The mechanism of binding mode and interaction of molecules with the COX-2 [PDB ID: 3LN1] has been scrutinized to understand the effectiveness of the molecules as anti-inflammatory agents. As we are searching for multi-targeted drugs, molecules were also expected to have antimicrobial and anti-oxidant activities.

Experimental

Materials and methods

Laboratory-grade chemicals and standard techniques were utilized to undertake the designed reactions. Melting points of the aimed compounds were determined via the open capillary method. The compounds synthesized were confirmed and characterized with the aid of thin-layer chromatography (TLC), AT-IR, ^{1}H NMR and ^{13}C NMR data with the help of a Bruker DRX-300 (400 MHz NMR) and Bruker DRX-75 (100 MHz NMR) spectrometer in (DMSO)-d_6, respectively, using tetramethylsilane (TMS; an internal standard) and LC mass spectra by a Shimadzu LCMS 2010 spectrometer.

1-(4-(2,4-Dichlorobenzyloxy)-3-methoxybenzylidene)thiosemicarbazide ***(9b)***

Creamy solid; Yield: 82%; FT IR (ATR, ν_{max}, cm^{-1}): 3381 and 3356 (N–H_2), 3251 (N–H), 3104 (Ar–H), 2923, 2871 (C–H), 1615 (C=N), 1579 (C=C), 1247 (C–N), 1089 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.80 (s, 3H, –OCH_3), 5.12 (s, 2H, –OCH_2), 7.02 (d, 2H, $J = 8.0$ Hz, C_5–H of 3-OCH_3-C_6H_3O), 7.12 (dd, 1H, $J = 1.6$ and 8.0 Hz, C_6–H of 3–OCH_3–C_6H_3O), 7.46 (dd, 1H, $J = 2.4$ and 8.8 Hz, C_5–H of 2,4-Cl_2-C_7H_3), 7.52 (d, 1H, $J = 1.6$ Hz, C_3–H of 3-OCH_3-C_6H_3O), 7.57 (d, 1H, $J = 8.0$ Hz, C_6–H of 2,4-Cl_2-C_7H_3), 7.65 (d, 1H, $J = 2.0$ Hz, C_3–H of 2,4-Cl_2-C_7H_3), 7.94 (s, 1H, =C–H), 7.99 (bs, 1H, NH_2), 8.14 (bs, 1H, NH_2), 11.30 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 178.1, 150.1, 149.6, 142.8,

134.0, 134.1, 133.9, 131.8, 129.3, 128.3, 128.0, 122.4, 113.7, 109.6, 67.4, 56.3; ESI–MS: 383.98 $(M + H)^+$, 385.94 $[(M + H)^+ + 2]$, 388.03 $[(M + H)^+ + 4]$.

1-(4-(2-Fluorobenzyloxy)-3-methoxybenzylidene)thiosemicarbazide ***(9c)***

Creamy solid; Yield: 81%; FT IR (ATR, ν_{max}, cm^{-1}): 3452 and 3332 ($N–H_2$), 3249 (N–H), 3120 (Ar–H), 2947, 2827 (C–H), 1609 (C=N), 1543 (C=C), 1292 (C–N), 1169 (C–F), 1110 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.94 (s, 3H, $–OCH_3$), 5.26 (s, 2H, $–OCH_2$), 7.09 (d, 1H, $J = 8.0$ Hz, C_5–H of 3-OCH_3-C_6H_3O), 7.15 (dd, 1H, $J = 1.6$ and 8.8 Hz, C_6–H of 3-OCH_3-C_6H_3O), 7.24 (d, 1H, $J = 2.0$ Hz, C_2–H of 3-OCH_3-C_6H_3O), 7.37–7.41 (m, 1H, C_3–H of 2- F-C_7H_6), 7.50–7.53 (m, 1H, C_5–H of 2- F-C_7H_6), 7.54–7.57 (m, 1H, C_6–H of 2- F-C_7H_6), 7.61–7.65 (m, 1H, C_4–H of 2-F-C_7H_6), 7.92 (s, 1H, =C–H), 7.98 (bs, 1H, NH_2), 8.19 (bs, 1H, NH_2), 11.29 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 178.1, 159.63 (d, C-2, $J_{C,F} = 245.1$ Hz), 149.6–132.2 (Ar–C), 132.1 (d, C-6, $J_{C,F} = 3.8$ Hz), 130.6 (d, C-5, $J_{C,F} = 8.3$ Hz), 125.1 (d, C-1, $J_{C,F} = 3.0$ Hz), 124.2 (d, C-4, $J_{C,F} = 14.4$ Hz), 115.5 (d, C-3, $J_{C,F} = 21.3$ Hz), 129.3, 113.7, 109.6, 67.4; ESI–MS: 334.09 $(M + H)^+$.

1-((5-Bromothiophen-2-yl)methylene)thiosemicarbazide ***(9d)***

Creamy yellow solid; Yield: 86%; FT IR (ATR, ν_{max}, cm^{-1}): 3426 and 3273 ($N–H_2$), 3156 (N–H), 3028 (Ar–H), 2999, 2966 (C–H), 1607 (C=N), 1591 (C=C), 1279 (C–N), 1090 (C=S), 559 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.23 (d, 1H, $J = 4.0$ Hz, thiophene-H), 7.27 (d, 1H, $J = 4.0$ Hz, thiophenyl-H), 7.63 (bs, 1H, NH_2), 8.14 (s, 1H, =C–H), 8.21 (bs, 1H, NH_2), 11.49(s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 177.6, 140.6, 136.6, 131.2, 131.09, 114.5; ESI–MS: 263.92 $(M + H)^+$, 266.19 $[(M + H)^+ + 2]$.

1-(4-(Piperidin-1-yl)benzylidene)thiosemicarbazide ***(9e)***

Greenish-yellow solid; Yield: 86%; FT IR (ATR, ν_{max}, cm^{-1}): 3452 and 3332 ($N–H_2$), 3249 (N–H), 3120 (Ar–H), 2947, 2827 (C–H), 1609 (C=N), 1110 (C=S), 1534 (C=C), 1061 (C–N); ^{1}H NMR (400 MHz, DMSO-d_6, $\underline{\delta}$ ppm): 1.56 (s, 6H, piperidyl-H), 3.25 (s, 4H, piperidyl-H), 6.90 (d, 2H, $J = 9.2$ Hz, C_2 and $C_{2'}$ of C_6H_4), 7.58 (d, 2H, $J = 8.8$ Hz, C_3 and $C_{3'}$ of C_6H_4), 7.78 (bs, 1H, NH_2), 7.94 (s, 1H, =C–H), 8.01 (bs, 1H, NH_2), 11.21(s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 177.2, 152.3, 143.1, 128.5, 123.2, 114.5, 48.4, 24.9, 23.9; ESI–MS: 263.10 $(M + H)^+$.

1-(4-Morpholinobenzylidene)thiosemicarbazide ***(9f)***

Orange-red crystals; Yield: 80%; FT IR (ATR, ν_{max}, cm^{-1}): 3456 and 3314 ($N–H_2$), 3296 (N–H), 3068 (Ar–H), 2966, 2875 (C–H), 1615 (C=N), 1560 (C=C), 1110 (C–O–C), 1101 (C–N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.09-3.11 (m, 4H, morpholine-CH_2), 3.57–3.69 (m, 4H, morpholine-CH_2), 6.84 (d, 2H, $J = 9.2$ Hz, C_2 and $C_{2'}$ of C_6H_4), 7.56 (d, 2H, $J = 8.8$ Hz, C_3 and $C_{3'}$ of C_6H_4), 7.75 (bs, 1H, NH_2), 7.90 (s, 1H, =C–H), 8.00 (bs, 1H, NH_2), 11.19 (s, 1H, N–H); ^{13}C NMR (100 MHz,

DMSO-d_6, δ ppm): 177.6, 153.0, 142.9, 129.1, 114.1, 66.5, 48.3, 25.0; ESI–MS: 264.92 $(M + H)^+$.

General procedure for the synthesis of **11a–r** is in the supplementary data.

*1-(4-(2,4-Dichlorobenzyloxy)benzylidene)-2-(4-p-tolylthiazol-2-yl)hydrazine **(11a)***

Creamy solid; Yield: 86%; MP: 178–180 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3448 (N–H), 3067 (Ar–H), 2920, 2727 (C–H), 1620 (C=N), 1566 (C=C), 1099 (C–N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.46 (s, 3H, $–CH_3$), 5.16 (s, 2H, $-OCH_2$), 7.07 (d, 2H, $J = 8.8$ Hz, C_3 and $C_{3'}$–H of C_6H_4O), 7.17–7.19 (m, 3H, thiazolyl-H, C_3 and $C_{3'}$–H of 4-CH_3-C_6H_4), 7.45–7.47 (dd, 1H, $J = 2.0$ and 8.0 Hz, C_5–H of 2,4-Cl_2-C_7H_3), 7.57–7.61 (m, 3H, C_6–H of $C_7H_5Cl_2$ and $C_2{}'$ $C_{2'}$ of 4-CH_3-C_6H_4), 7.67 (d, 1H, $J = 2.0$ Hz, C_3–H of 2,4-Cl_2-C_7H_3), 7.70 (d, 2H, $J = 8.0$ Hz, C_2 and $C_{2'}$–H of C_6H_4O), 7.97 (s, 1H, =C–H), 11.91 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.7, 159.4, 141.8, 133.8, 132.8, 102.9, 66.9, 21.2; ESI–MS of $C_{24}H_{19}N_3Cl_2$-OS: 467.96 $(M + H)^+$, 469.95 $[(M + H)^+ + 2]$, 471.95 $[(M + H)^+ + 4]$.

*1-(4-(2,4-Dichlorobenzyloxy)benzylidene)-2-(4-(2,4-dichlorophenyl)thiazol-2-yl)hydrazine **(11c)***

Creamy solid; Yield: 88%; MP: 172–174 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3250 (N–H), 3073 (Ar–H), 2926, 2866 (C–H), 1699 (C=N), 1570 (C=C), 1096 (C–N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.15 (s, 2H, $-OCH_2$), 7.06 (d, 2H, $J = 8.8$ Hz, C_3 and $C_{3'}$–H of C_6H_4O), 7.34 (s, 1H, thiazolyl-H), 7.43–7.48 (m, 2H, C_3–H and C_5–H of 2,4-Cl_2-C_7H_3), 7.58–7.65 (m, 5H, Ar–H), 7.85 (d, 1H, $J = 8.8$ Hz, C_6–H of 2,4-Cl_2-C_6H_3), 8.04 (s, 1H, =C–H), 12.04 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.9, 159.6, 142.6, 134.0, 133.7, 133.1, 132.7, 132.2, 131.8, 130.1, 129.4, 128.5, 128.1, 128.0, 127.9, 115.6, 109.4, 66.9; ESI–MS of $C_{23}H_{15}N_3Cl_4OS$: 521.98 $(M + H)^+$, 523.89 $[(M + H)^+ + 2]$, 525.94 $[(M + H)^+ + 4]$, 527.79 $[(M + H)^+ + 6]$, 530.01 $[(M + H)^+ + 8]$.

*1-(4-(2,4-Dichlorobenzyloxy)-3-methoxybenzylidene)-2-(4-p-tolylthiazol-2-yl)hydrazine **(11d)***

Creamy solid; Yield: 90%; MP: 98–100 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3172 (N–H), 3080 (Ar–H), 2923, 2854 (C–H), 1623 (C=N), 1565 (C=C), 1097 (C–N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.28 (s, 3H, $-CH_3$), 4.31 (s, 1H, $–OCH_3$), 5.12 (s, 2H, $-OCH_2$), 7.03 (d, 1H, $J = 8.4$ Hz, C_5–H of 3-OCH_3–C_6H_3O), 7.12 (dd, 1H, $J = 2.0$ and 8.0 Hz, C_6–H of 3-OCH_3-C_6H_3O), 7.19 (s, 1H, thiazolyl-H), 7.46 (dd, 1H, $J = 2.0$ and 8.0 Hz, C_5–H of 2,4-Cl_2-C_7H_3), 7.53 (d, 1H, $J = 2.0$ Hz, C_2–H of 3-OCH_3-C_6H_3O), 7.55-7.59 (m, 3H, C_6–H of 2,4-Cl_2-C_7H_3 and C_3,$C_{3'}$ of 4-CH_3-C_6H_4), 7.66 (s, 1H, C_3–H of 2,4-Cl_2-C_7H_3), 7.69 (d, 2H, $J = 8.4$ Hz, C_2,$C_{2'}$ of 4-CH_3-C_6H_4), 7.91 (s, 1H, =C–H), 12.01 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.7, 159.4, 141.8, 133.8, 132.8, 102.9, 66.9, 21.2; ESI–MS of $C_{24}H_{19}N_3Cl_2OS$: 497.96 $(M + H)^+$, 499.96 $[(M + H)^+ + 2]$, 501.96 $[(M + H)^+ + 4]$.

1-(4-(2,4-Dichlorobenzyloxy)-3-methoxybenzylidene)-2-(4-(4-bromophenyl)thiazol-2-yl) hydrazine ***(11e)***

Creamy solid; Yield: 86%; MP: 166–168 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3178 (N–H), 3077 (Ar–H), 2924, 2854 (C–H), 1624 (C=N), 1566 (C=C), 1099 (C–N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.33 (s, 1H, -OCH_3), 5.13 (s, 2H, -OCH_2), 7.06 (d, 1H, $J = 8.8$ Hz, C_5–H of 3-OCH_3-C_6H_3O), 7.14 (dd, 1H, $J = 1.6$ and 8.4 Hz, C_6–H of 3-OCH_3-C_6H_3O), 7.27 (d, 1H, $J = 1.6$ Hz, C_2–H of 3-OCH_3-C_6H_3O), 7.35 (s, 1H, thiazolyl-H), 7.46 (dd, 1H, $J = 1.6$ and 8.4 Hz, C_5–H of 2,4-Cl_2-C_7H_3), 7.55–7.59 (m, 3H, C_6–H of 2,4-Cl_2-C_7H_3 and C_2, $C_{2'}$–H of 4-Br-C_6H_4), 7.65 (d, 1H, $J = 1.6$ Hz, C_3–H of 2,4-Cl_2-C_7H_3), 7.77 (d, 2H, $J = 8.8$ Hz, C_3,$C_{3'}$–H of p-Br-C_6H_4), 7.95 (s, 1H, =C–H), 12.24 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.9, 114.3, 109.5, 104.8, 67.5, 56.0; ESI–MS of $C_{24}H_{18}N_3$-$BrCl_2O_2S$: 561.84 $(M + H)^+$, 563.84 $[(M + H)^+ + 2]$, 565.83 $[(M + H)^+ + 4]$, 566.83 $[(M + H)^+ + 6]$.

1-(4-(2,4-Dichlorobenzyloxy)-3-methoxybenzylidene)-2-(4-(2,4-dichlorophenyl)thiazol-2-yl) hydrazine ***(11f)***

Creamy solid; Yield: 89%; MP: 176–178 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3180 (N–H), 3081 (Ar–H), 2926, 2857 (C–H), 1625 (C=N), 1568 (C=C), 1089 (C–N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.81 (s, 1H, –OCH_3), 5.14 (s, 2H, -OCH_2), 7.07 (d, 1H, $J = 8.4$ Hz, C_5–H of 3-OCH_3-C_6H_3O), 7.16 (dd, 1H, $J = 2.0$ and 8.0 Hz, C_6–H of 3-OCH_3-C_6H_3O), 7.29 (d, 1H, $J = 1.6$ Hz, C_2–H of 3-OCH_3-C_6H_3O), 7.35 (s, 1H, thiazolyl-H), 7.45–7.49 (m, 2H, C_5–H of 2,4-Cl_2-C_7H_3 and $C_{5'}$–H of C_6H_3), 7.59 (d, 2H, $J = 8.4$ Hz, C_6–H of 2,4-Cl_2-C_7H_3), 7.65 (d, 2H, $J = 2.4$ Hz, C_3–H of 2,4-Cl_2-C_7H_3 and $C_{3'}$–H of C_6H_3), 7.87 (d, 1H, $J = 8.0$ Hz, $C_{6'}$–H of 2,4-Cl_2-C_7H_3), 7.99 (s, 1H, =C–H), 12.23 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.9, 149.9, 149.2–120.5, 114.2, 109.6, 109.4, 67.5, 56.0; ESI–MS of $C_{24}H_{17}N_3Cl_4O_2S$: 551.85 $(M + H)^+$, 553.93 $[(M + H)^+ + 2]$, 555.79 $[(M + H)^+ + 4]$, 557.83 $[(M + H)^+ + 6]$, 559.90 $[(M + H)^+ + 8]$.

1-(4-(2-Fluorobenzyloxy)-3-methoxybenzylidene)-2-(4-p-tolylthiazol-2-yl)hydrazine ***(11g)***

Creamy solid; Yield: 84%; MP: 138–140 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3421 (N–H), 3071 (Ar–H), 2928, 2862 (C–H), 1620 (C=N), 1512 (C=C), 1099 (C–N), 759 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.39 (s, 1H, -CH_3), 3.95 (s, 1H, -OCH_3), 5.27 (s, 2H, -OCH_2), 6.65 (s, 1H, C_2–H of 3-OCH_3-C_6H_3O), 6.95 (d, 2H, $J = 8.4$ Hz, C_3–H and $C_{3'}$–H of 4-CH_3-C_6H_4), 7.07 (d, 1H, $J = 8.4$ Hz, C_5–H of 3-OCH_3-C_6H_3O), 7.11–7.17 (m, 3H, C_3–H and C_5–H of 2-F-C_7H_6 and C_6–H of 3-OCH_3-C_6H_3O), 7.25 (s, 1H, thiazolyl-H), 7.27–7.31 (m, 1H, C_6–H of 2-F-C_7H_6), 7.48–7.51 (m, 1H, C_4–H of 2-F-C_7H_6), 7.59 (d, 2H, $J = 8.0$ Hz, C_2–H and $C_{2'}$–H of 4-CH_3-C_6H_4), 8.17 (s, 1H, =C–H), 11.77 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.9, 160.8 (d, C-2 $J_{C,F} = 238$ Hz), 149.6–132.1 (Ar–C), 131.2 (d, C-6, $J_{C,F} = 10.0$ Hz), 130.8 (d, C-5 $J_{C,F} = 10.0$ Hz), 124.9 (d, C-1 $J_{C,F} = 10.0$ Hz), 124.0 (d, C-4 $J_{C,F} = 20.0$ Hz),

115.7 (d, C-3 $J_{C,F} = 20.0$), 130.0, 128.0, 127.7, 120.4, 114.0, 109.5, 109.2, 64.5, 56.0; ESI–MS of $C_{25}H_{22}N_3FO_2S$: 448.04 $(M + H)^+$.

1-(4-(2-Fluorobenzyloxy)-3-methoxybenzylidene)-2-(4-(4-bromophenyl)thiazol-2-yl) hydrazine ***(11h)***

Creamy solid; Yield: 86%; MP: 156–158 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3231 (N–H), 3047 (Ar–H), 2968, 22941 (C–H), 1620 (C = N), 1595 (C=C), 1099 (C–N), 758 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.97 (s, 1H, -OCH_3), 5.29 (s, 2H, -OCH_2), 7.10 (d, 1H, $J = 8.0$ Hz, C_5–H of 3-OCH_3-C_6H_3O), 7.17 (dd, 1H, $J = 1.6$ and 8.4 Hz, C_6–H of 3-OCH_3-C_6H_3O), 7.20–7.25 (m, 2H, C_3–H of 2-F-C_7H_6 and C_5–H of 2-F-C_7H_6), 7.28 (d, 1H, $J = 2.0$ Hz, C_2–H of 3-OCH_3-C_6H_3O), 7.35 (s, 1H, thiazolyl-H), 7.37–7.41 (m, 1H, C_6–H of 2-F-C_7H_6), 7.51–7.54 (m, 1H, C_4–H of 2-F-C_7H_6), 7.59 (d, 2H, $J = 8.0$ Hz, C_2, $C_{2'}$–H of 4-Br-C_6H_4), 7.79 (d, 2H, $J = 8.4$ Hz, C_3, $C_{3'}$–H of 4-Br-C_6H_4), 8.01 (s, 1H, =C–H), 11.86 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 169.2, 161.4 (d, C-2 $J_{C,F} = 240$ Hz,), 149.6–132.1 (Ar–C), 131.2 (d, C-6, $J_{C,F} = 10.0$ Hz), 130.8 (d, C-5 $J_{C,F} = 10.0$ -Hz), 125.0 (d, C-1 $J_{C,F} = 3.0$ Hz), 124.0 (d, C-4 $J_{C,F} = 21.0$ Hz), 115.8 (d, C-3 $J_{C,F} = 21.1$), 130.0, 128.0, 127.7, 120.4, 114.0, 109.5, 109.2, 64.5, 56.0; ESI–MS of $C_{24}H_{19}N_3BrFO_2S$: 512.05 $(M + H)^+$, 514.12 $[(M + H)^+ + 2]$.

1-(4-(2-Fluorobenzyloxy)-3-methoxybenzylidene)-2-(4-(2,4-dichlorophenyl)thiazol-2-yl) hydrazine ***(11i)***

Creamy solid; Yield: 84%; MP: 197–199 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3343 (N–H), 3070 (Ar–H), 2941, 2882 (C–H), 1626 (C=N), 1576 (C=C), 1033 (C–N), 1138 (C-F), 760 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.78 (s, 1H, -OCH_3), 5.12 (s, 2H, -OCH_2), 7.11 (d, 1H, $J = 8.8$ Hz, C_5–H of 3-OCH_3-C_6H_3O), 7.16 (dd, 1H, $J = 1.6$ and 8.8 Hz, C_6–H of 3-OCH_3-C_6H_3O), 7.19–7.24 (m, 2H, C_3–H of 2-F-C_7H_6 and C_5–H of 2-F-C_7H_6), 7.27 (s, 1H, C_2–H of 3-OCH_3-C_6H_3O), 7.33 (s, 1H, thiazolyl-H), 7.36–7.40 (m, 1H, C_6–H of 2-F-C_7H_6), 7.46 (dd, 1H, $J = 2.0$ and 8.4 Hz, C_5–H of 2,4-Cl_2-C_6H_3), 7.50–7.52 (m, 1H, C_4–H of 2-F-C_7H_6), 7.65 (d, 1H, $J = 2.4$ Hz, C_3–H of 2,4-Cl_2-C_6H_3), 7.85 (d, 1H, $J = 8.8$ Hz, C_6–H of 2,4-Cl_2-C_6H_3), 8.02 (s, 1H, =C–H), 11.79 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.9, 160.9 (d, C-2, $J_{C,F} = 245.1$ Hz), 149.5–132.2 (Ar–C), 131.3 (d, C-6, $J_{C,F} = 3.8$ Hz), 130.9 (d, C-5, $J_{C,F} = 8.3$ Hz), 125.0 (d, C-1, $J_{C,F} = 3.0$ Hz), 124.2 (d, C-4, $J_{C,F} = 14.4$ Hz), 115.8 (d, C-3, $J_{C,F} = 21.3$ Hz), 130.1, 128.2, 127.9, 120.6, 114.1, 109.6, 109.4, 64.7, 56.0; $C_{24}H_{18}N_3Cl_2FO_2S$: 501.93 $(M + H)^+$, 503.93 $[(M + H)^+ + 2]$, 505.93 $[(M + H)^+ + 4]$.

1-((5-Bromothiophen-2-yl)methylene)-2-(4-4-tolylthiazol-2-yl)hydrazine ***(11j)***

Creamy solid; Yield: 90%; MP: 180–181 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3227 (N–H), 3053 (Ar–H), 2963, 2932 (C–H), 1574 (C=C), 1052 (C–N), 557 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.04 (s, 3H, -CH_3), 7.18 (d, 1H, $J = 4.0$ Hz, C_3–H of 5-Br-thiophene ring), 7.20 (d, 1H, $J = 3.6$ Hz, C_4–H of 5-Br- thiophene

ring), 7.35 (s, 1H, thiazolyl-H), 7.58 (d, 2H, $J = 8.4$ Hz, C_3 and $C_{3'}$–H of 4-CH_3-C_6H_4), 7.77 (d, 2H, $J = 8.0$ Hz, C_2 and $C_{2'}$–H of 4-CH_3-C_6H_4), 8.04 (s, 1H, =C–H), 12.16 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.74, 140.9, 135.4, 131.1, 130.5, 129.0, 127.0, 120.2, 113.1, 104.3; $C_{15}H_{12}N_3BrS_2$: 377.92 $(M + H)^+$, 379.89 $[(M + H)^+ + 2]$.

2-(4-(4-Bromophenyl)thiazol-2-yl)-1-((5-bromothiophen-2-yl)methylene)hydrazine ***(11k)***

Greenish crystalline solid; Yield: 90%; MP: 184–186 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3261 (N–H), 3086 (Ar–H), 3016, 2955 (C–H), 1692 (C = N), 1595 (C=C), 1092 (C–N), 554 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.23 (d, 1H, $J = 4.0$ Hz, C_3–H of 5-Br- thiophene ring), 7.20 (d, 1H, $J = 3.6$ Hz, C_4–H of 5-Br-thiophene ring), 7.40 (s, 1H, thiazolyl-H), 7.60 (d, 2H, $J = 8.8$ Hz, C_3 and $C_{3'}$–H of 4-Br-C_6H_4), 7.80 (d, 2H, $J = 8.4$ Hz, C_2 and $C_{2'}$–H of 4-Br-C_6H_4), 8.14 (s, 1H, =C–H), 12.23 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.8, 141.0, 135.9, 131.5, 131.2, 129.5, 127.5, 120.5, 113.3, 104.8; $C_{14}H_9N_3Br_2S_2$: 441.91 $(M + H)^+$, 443.94 $[(M + H)^+ + 2]$, 445.89 $[(M + H)^+ + 4]$.

1-((5-Bromothiophen-2-yl)methylene)-2-(4-(2,4-dichlorophenyl)thiazol-2-yl)hydrazine ***(11l)***

Greanish solid; Yield: 89%; MP: 138–140 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3379 (N–H), 3186 (Ar–H), 3065, 2953 (C–H), 1626 (C = N), 1578 (C=C), 1074 (C–N), 797 (C–Cl), 559 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.22 (d, 1H, $J = 3.6$ Hz, C_3–H of 5-Br- thiophene ring), 7.25 (d, 1H, $J = 3.6$ Hz, C_4–H of 5-Br-thiophene ring), 7.39 (s, 1H, thiazolyl-H), 7.50 (dd, 1H, $J = 2.4$ and 8.4 Hz, C_5–H of 2,4-Cl_2-C_6H_3), 7.66 (d, 1H, $J = 2.0$ Hz, C_3–H of 2,4-Cl_2-C_6H_3), 7.87 (d, 1H, $J = 8.4$ Hz, C_6–H of 2,4-Cl_2-C_6H_3), 8.15 (s, 1H, =C–H), 12.19 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.0, 141.2, 140.1, 131.6, 131.4, 129.7, 127.8, 120.5, 113.5, 104.9; $C_{14}H_9N_3Br_2S_2$: 431.94 $(M + H)^+$, 433.87 $[(M + H)^+ + 2]$, 435.91 $[(M + H)^+ + 4]$, 437.96 $[(M + H)^+ + 6]$.

1-(4-(Piperidin-1-yl)benzylidene)-2-(4-4-tolylthiazol-2-yl)hydrazine ***(11m)***

Brown solid; Yield: 75%; MP: 168–170 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3343 (N–H), 3064 (Ar–H), 2953, 2874 (C–H), 1619 (C = N), 1564 (C=C), 1067 (C–N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.60–1.72 (m, 6H, pipiridine-CH_2), 2.28 (s, 3H, -CH_3), 3.29–3.40 (m, 4H, pipiridine-CH_2), 6.98 (d, 2H, $J = 8.4$ Hz, C_3, $C_{3'}$–H of C_6H_4), 7.15 (d, 2H, $J = 8.8$ Hz, C_3, $C_{3'}$–H of 4-CH_3-C_6H_4), 7.29 (d, 2H, $J = 8.4$ Hz, C_2, $C_{2'}$–H of C_6H_4), 7.30 (s, 1H, thiazolyl-H), 7.57 (d, 2H, $J = 8.4$ Hz, C_2, $C_{2'}$–H of 4-CH_3-C_6H_4), 7.98 (s, 1H, =C–H), 11.71 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.2, 149.1, 133.6, 131.0, 127.4, 127.3, 120.1, 104.2, 24.3, 24.2; $C_{22}H_{24}N_4S$: 377.14 $(M + H)^+$.

1-(4-(Piperidin-1-yl)benzylidene)-2-(4-(4-bromophenyl)thiazol-2-yl)hydrazine ***(11n)***

Brown solid; Yield: 78%; MP: 206–208 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3351 (N–H), 3069 (Ar–H), 2955, 2875 (C–H), 1622 (C = N), 1567 (C=C), 1070 (C–N), 557 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.62–1.74 (m, 6H, pipiridine-CH_2), 3.31–3.40 (m, 4H, pipiridine-CH_2), 7.18 (d, 2H, J = 8.4 Hz, C_3, $C_{3'}$–H of C_6H_4), 7.30 (d, 2H, J = 8.0 Hz, C_2, $C_{2'}$–H of C_6H_4), 7.31 (s, 1H, thiazolyl-H), 7.63 (d, 2H, J = 8.8 Hz, C_2 and $C_{2'}$–H of 4-Br-C_6H_4), 7.80 (d, 2H, J = 8.4 Hz, C_3 and $C_{3'}$–H of 4-Br-C_6H_4) 8.00 (s, 1H, =C–H), 11.89 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.4, 149.3, 133.8, 131.5, 131.2, 127.6, 127.5, 120.5, 104.4, 24.4, 24.3; $C_{21}H_{21}N_4BrS$: 440.97 $(M + H)^+$, 442.96 $[(M + H)^+ + 2]$.

1-(4-(Piperidin-1-yl)benzylidene)-2-(4-(2,4-dichlorophenyl)thiazol-2-yl)hydrazine ***(11o)***

Brown solid; Yield: 80%; MP: 180–182 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3332 (N–H), 3071 (Ar–H), 2973, 2884 (C–H), 1622 (C = N), 1569 (C=C), 1082 (C–N), 793 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.63–1.73 (m, 6H, pipiridine-CH_2), 3.30–3.39 (m, 4H, pipiridine-CH_2), 7.18 (d, 2H, J = 8.4 Hz, C_3, $C_{3'}$–H of C_6H_4), 7.30 (d, 2H, J = 8.8 Hz, C_2, $C_{2'}$–H of C_6H_4), 7.32 (s, 1H, thiazolyl-H), 7.46 (dd, 1H, J = 2.0 and 8.4 Hz, C_5–H of 2,4-Cl_2-C_6H_3), 7.64 (d, 1H, J = 2.4 Hz, C_3–H of C_6H_4), 7.86 (d, 1H, J = 8.4 Hz, C_6–H of 2,4-Cl_2-C_6H_3), 8.00 (s, 1H, =C–H), 11.74 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.4, 149.4, 133.7, 131.3, 127.5, 127.3, 120.3, 104.3, 24.3, 24.1; $C_{21}H_{20}N_4Cl_2S$: 431.12 $(M + H)^+$, 433.23 $[(M + H)^+ + 2]$, 435.18 $[(M + H)^+ + 4]$.

1-(4-Morpholinobenzylidene)-2-(4-4-tolylthiazol-2-yl)hydrazine ***(11p)***

Brown solid; Yield: 76%; MP: 226–228 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3298 (N–H), 3070 (Ar–H), 2965, 2874 (C–H), 1612 (C = N), 1559 (C=C), 1109 (C–O–C), 1099 (C–N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.27 (s, 3H, -CH_3), 3.13–3.15 (m, 4H, morpholine-CH_2), 3.69–3.73 (m, 4H, morpholine-CH_2), 6.96 (d, 2H, J = 8.0 Hz, C_3, $C_{3'}$–H of C_6H_4), 7.16 (d, 2H, J = 8.8 Hz, C_3, $C_{3'}$–H of 4-CH_3-C_6H_4), 7.31 (d, 2H, J = 8.4 Hz, C_2, $C_{2'}$–H of C_6H_4), 7.33 (s, 1H, thiazolyl-H), 7.57 (d, 2H, J = 8.4 Hz, C_2 and $C_{2'}$–H of 4-CH_3-C_6H_4), 7.97 (s, 1H, =C–H), 11.91 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.9, 149.7, 142.3, 132.6, 132.5, 131.8, 130.0, 127.5, 125.1, 109.0, 66.1, 47.8; $C_{21}H_{22}N_4OS$: 378.95 $(M + H)^+$.

1-(4-Morpholinobenzylidene)-2-(4-(4-bromophenyl)thiazol-2-yl)hydrazine ***(11q)***

Brown solid; Yield: 77%; MP: 258–260 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3303 (N–H), 3075 (Ar–H), 2981, 2879 (C–H), 1626 (C = N), 1565 (C=C), 1124 (C–O–C), 1099 (C–N), 558 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.17–3.19 (m, 4H, morpholine-CH_2), 3.74–3.77 (m, 4H, morpholine-CH_2), 7.19 (d, 2H, J = 8.0 Hz, C_3, $C_{3'}$–H of C_6H_4), 7.32 (d, 2H, J = 8.0 Hz, C_2 and $C_{2'}$–H of C_6H_4), 7.34 (s, 1H,

thiazolyl-H), 7.63 (d, 2H, $J = 8.4$ Hz, C_3 and $C_{3'}$–H of 4-Br-C_6H_4), 7.81 (d, 2H, $J = 8.8$ Hz, C_2 and $C_{2'}$–H of 4-Br-C_6H_4), 8.01 (s, 1H, =C–H), 12.19 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.2, 152.3, 142.5, 133.1, 132.8, 132.5, 132.1, 130.4, 127.9, 125.5, 109.4, 66.5, 48.4; $C_{20}H_{19}N_4BrOS$: 443.13 $(M + H)^+$, 445.21 $[(M + H)^+ + 2]$.

1-(4-Morpholinobenzylidene)-2-(4-(2,4-dichlorophenyl)thiazol-2-yl)hydrazine ***(11r)***

Brown solid; Yield: 80%; MP: 248–250 °C; FT IR (ATR, ν_{max}, cm^{-1}): 3312 (N–H), 3080 (Ar–H), 2982, 2881 (C–H), 1619 (C=N), 1564 (C=C), 1112 (C–O–C), 1084 (C–N), 757 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.15–3.18 (m, 4H, morpholine-CH_2), 3.71–3.73 (m, 4H, morpholine-CH_2), 6.97 (d, 2H, $J = 8.8$ Hz, C_3 and $C_{3'}$–H of C_6H_4), 7.34 (s, 1H, thiazolyl-H), 7.46–7.51 (m, 3H, C_2 and $C_{2'}$–H of C_6H_4 and C_5–H of 2,4-Cl_2-C_6H_3), 7.66 (d, 1H, $J = 2.4$ Hz, C_3–H of 2,4-Cl_2-C_6H_3), 7.88 (d, 1H, $J = 8.8$ Hz, C_6–H of 2,4-Cl_2-C_6H_3), 7.92 (s, 1H, =C–H), 11.92 (s, 1H, N–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 168.0, 152.2, 142.5, 132.8, 132.7, 132.6, 132.0, 130.2, 127.9, 125.3, 115.0, 109.2, 66.4, 48.1; $C_{20}H_{18}N_4Cl_2OS$: 433.13 $(M + H)^+$, 435.14 $[(M + H)^+ + 2]$, 437.23 $[(M + H)^+ + 4]$.

Anti-inflammatory activity

In vivo anti-inflammatory activity of the synthesized compounds (**11a**–**r**) was studied by fabricating the inflammatory reaction with the help of irritants in the form of paw edema in Wistar albino rats weighing 150–250 g. Carrageenan-induced paw edema is the most frequently used experimental method [37, 38]. Carrageenan is a sulfated polysaccharide obtained from seaweed (Rhodophyceae) causing the release of histamine, 5-HT, bradykinin and prostaglandins which produce inflammation and edema.

The weighed Wistar albino rats were numbered and a mark was made on the left hind paw just beyond tibiotarsal junction so that every time the paw is dipped in the mercury column up to the fixed mark to ensure constant paw volume. After 1 h, 0.1 mL of 1% carrageenan suspension in isosaline solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured at an hourly interval for 5 h. The initial paw volume of each rat was noted by the mercury displacement method. The animals were divided into different groups, each containing 6 rats. The first group of rats was treated with carrageenan (control), the second group was administered with a dose of 20 mg/kg of Diclofenac (standard), and the suspension of test compounds (20 mg/kg) was injected into the remaining groups (the dose of the test compounds was fixed by conducting acute toxicity studies as per OECD 425 guidelines and there were no signs of toxicity at the given dose level). The paw volume of both legs was noted for 1 h, 2 h, and after 3 h carrageenan challenge. The mean paw edema value obtained for the test group is compared with its mean value of the control group.

Anti-inflammatory activity was measured as the percentage reduction in edema level when the drug was present, relative to control. Percentage inhibition was calculated by using the relationship,

$$\%\,\text{inhibition} = \{1 - Vt/Vc\} \times 100$$

where *Vt* is the edema volume in the drug-treated group and *Vc* is the edema volume in the control group.

Cytotoxicity

The test compounds were studied for short-term in vitro cytotoxicity using Dalton's lymphoma ascites cells (DLA) [39]. The tumor cells aspirated from the peritoneal cavity of tumor mice were washed twice with PBS or normal saline. Cell viability was determined by the trypan blue exclusion method. A viable cell suspension (1×10^6 cells in 0.1 mL) was added with various concentrations of the test compounds prepared (10, 20, 50, 100 and 200 μg/mL) and the volume was made up to 1 mL using phosphate-buffered saline (PBS). The control tube was with the cell suspension. These assay mixtures were incubated for 3 h at 37 °C. Further, the cell suspension was mixed with 0.1 mL of 1% trypan blue and kept for 2–3 min and loaded on a hemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The numbers of stained and unstained cells were counted separately using this equation:

$$\%\,\text{Cytotoxicity} = \frac{\text{No. of dead cells}}{\text{No. of live cells} + \text{No. of dead cells}} \times 100$$

Anti-oxidant activity

A free radical scavenging activity of the title compounds were tested as per the literature [36]. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) was procured from Sigma Aldrich. A 1-mL solution of 0.135 mM DPPH in methanol was prepared and was mixed with 1 mL of varying concentrations (0, 100, 200, 300, 400 and 500 μg/mL) of the test solutions. The reaction mixture prepared was vortexed properly and left in the dark for 30 min at room temperature. The purple color of DPPH generally vanishes when it undergoes reaction with an anti-oxidant present in the medium and hence the absorbance. The absorbance of the mixture was measured using ascorbic acid as the reference standard at 517 nm. Anti-oxidant property of the test compounds is calculated using the following formula:

$$\%\,\text{DPPH radical scavenging activity} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

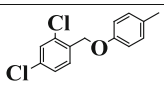
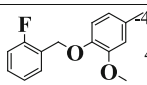
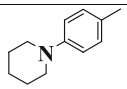
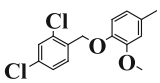
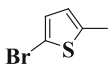
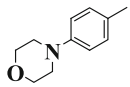
The anti-oxidant property of the test compound was demonstrated by IC_{50} values (50% inhibitory concentration) depending upon the percentage of DPPH radicals utilized in the reaction. Lower IC_{50} values indicate the highest anti-oxidant activity. Imax (maximum percentage inhibition) values for the compounds were also calculate using 'GraphPad Software' [40].

Antibacterial activity

The antibacterial activity of the novel compounds was determined using the literature [41]. Solutions were created by dissolving the test compounds in dimethyl sulfoxide (DMSO) and diluted with distilled water to get the concentration of 75 μg/mL under aseptic conditions. Standard drugs, Ciprofloxacin and Streptomycin, were procured from HIMEDIA were used to compare the activity of the synthesized molecules at 75 μg/mL. Nutrient Agar Media was prepared by dissolving 38 g of Mueller–Hinton agar with 1000 mL of distilled water and was sterilized by autoclaving at 121 °C for 20 min with 15 psi pressure. The prepared media were cooled at 45 °C with stirring and bacterial culture was inoculated onto the media and poured into Petri plates under aseptic condition. The plates were kept for solidification for 1 h. Then, 6-mm-diameter bores were made at equal distances using a sterile borer and were filled with the test solution. A zone of inhibition was measured after incubating the plates at 37 °C for 24 h.

Molecular docking

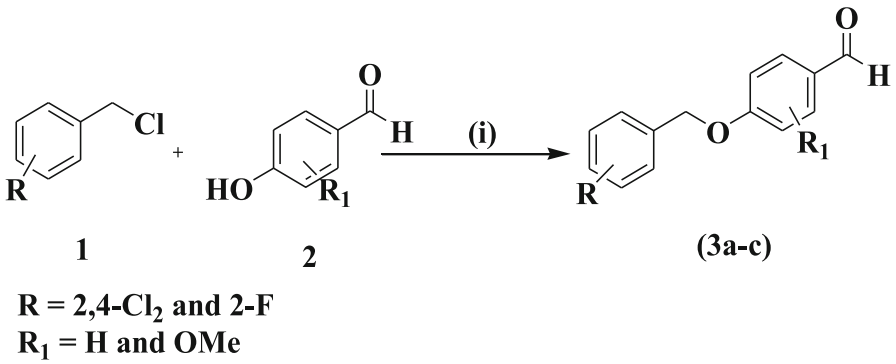
The binding interactions of the synthesized molecules with the COX-2 protein were assessed with the aid of molecular docking. The docking study was executed by means of a C-Dock suite. The crystallographic structure of the COX-2 protein with PDB ID: 3LN1 was collected from the RSCB protein Data Bank (www.rscb.org). The protein obtained was pre-processed by deleting all the chains of the protein except Chain-A, and heteroatoms and water molecules were also being deleted. With the help of the Chemistry tool, hydrogen atoms were added to the protein. Using the receptor–ligand interaction tool, protein was prepared for docking. A binding site in the protein was identified and utilized for docking, leaving the remaining part of the protein. Ligands drawn in Chemsketch were uploaded on Discovery studio 3.5 and were prepared with the help of the Small molecule tool. Docking has been carried out to understand the receptor–ligand interactions in producing the desired activity.

Compound	Ar	R_2	Compound	Ar	R_2	Compound	Ar	R_2
11a		-4-CH_3	**11g**		-4-CH_3	**11m**		-4-CH_3
11b		4-Br	**11h**		4-Br	**11n**		4-Br
11c		2,4-Cl_2	**11i**		2,4-Cl_2	**11o**		2,4-Cl_2
11d		-4-CH_3	**11j**		-4-CH_3	**11p**		-4-CH_3
11e		4-Br	**11k**		4-Br	**11q**		4-Br
11f		2,4-Cl_2	**11l**		2,4-Cl_2	**11r**		2,4-Cl_2

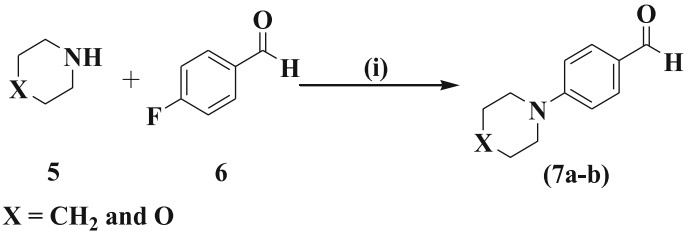
Results and discussion

Chemistry

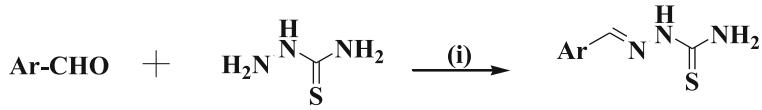
In the current exploratory study, target compounds were synthesized, as depicted in Schemes 1, 2, 3, and 4. The condensation of thiosemicarbazide with 4-(Benzyloxy)benzaldehydes (**3a–c**), 5-bromothiophene-2-carbaldehyde (**4**), 4-(piperidin-1-yl)benzaldehyde (**7a**) and 4-morpholinobenzaldehyde (**7b**) afforded the respective thiosemicarbazones (**9a–f**) as reported in the literature [42]. Compounds (**3a–c**) have been synthesized according to the procedure given by Bhattarai et al. [43]. The nucleophilic substitution of fluorine in the compound (**6**) with cyclic amines resulted in the formation of (**7a**) and (**7b**), respectively [44]. Thiosemicarbazones (**9a–f**) were made to react with respective phenacyl bromides and chlorides to get the desired molecules (**11a–r**) [2]. Completion of the reaction was monitored using TLC, recrystallization of the product was carried out using ethanol to afford the pure product. Formation of the new compounds was confirmed by spectral techniques like Attenuated Total Reflection–Fourier Transform Infrared (ATR–FTIR), Proton and ^{13}Carbon Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR), and Mass (MS). Physical parameters of the novel derivatives have been provided in the spectral section.



Scheme 1 Synthesis of 4-(benzyloxy)benzaldehydes. Reagents and condition: (i) K_2CO_3, DMF, reflux, 6 h

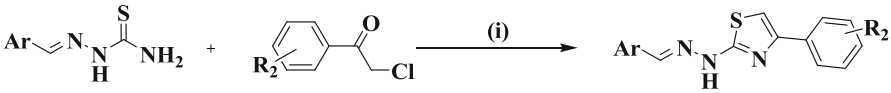


Scheme 2 Synthesis of compounds (**7a–b**). Reagents: (i) K_2CO_3, Acetone, Reflux for 8 h



(3a-c), 4, (7a-b) 8 (9a-f)

Scheme 3 Synthesis of substituted thiosemicarbazones (**9a–f**). Reagents: (i) EtOH, AcOH, 6 h



(9a-f) 10 (11a-r)

Scheme 4 Synthesis of the target compounds (**11a–r**). Reagents: EtOH, Reflux for 8 h

The formation of intermediates, thiosemicarbazones (**9a–f**) was supported by the IR (ATR) spectral data. The characteristic bands for NH_2 and C=S supported the formation of the products. For the prototype compound (**9a**), two characteristic absorption bands at 3379 and 3354 cm^{-1} were discerned for NH_2, and the occurrence of two strong bands near 1618 and 1086 cm^{-1} affirmed the presence of C=N and C=S groups, respectively. As in the case of IR, ^{1}H NMR also gave evidence for the thiosemicarbazone formation (**9a**) by a typical singlet for an exocycli C=C–H at δ 7.97 ppm. In addition to this, a doublet with $J = 9.2$ Hz was identified at δ 7.72 ppm for a proton on C_6 of the 2,4-Cl_2-C_7H_5 ring. A doublet at δ 7.63 ppm ($J = 2.0$ Hz) was identified for a C_3–H of 2,4-Cl_2-C_7H_5 due to meta-coupling. A doublet of doublet was observed at δ 7.43 ppm ($J = 2.4$ and 8.4 Hz) for C_5–H of the 2,4-Cl_2-C_7H_5 ring. The spectrum also showed two doublets at δ 7.02 and 7.58 ppm with $J = 8.8$ and 8.0 Hz, respectively, for the four protons of the benzylidine ring. A singlet for –benzyl (OCH_2) protons was assigned at δ 5.14 ppm. In the spectrum, two broad singlets were observed due to tautomerization of free N–H_2 at δ 7.99 and 8.14 ppm, respectively. The N–H group has given a singlet at δ 11.31 ppm. Characteristic peaks at δ 178.2 ppm (C=S), δ 159.8 ppm (–O–C=C–), δ 142.5 ppm (–C=N), δ 66.9 ppm (–O–CH_2) in the ^{13}C NMR spectrum ratified the product formation. Mass spectrum confirmed the product (**9a**) by giving isotopic peaks at 354.1 $(M + H)^+$, 355.94 $[(M + H)^+ + 2]$ and 358.02 $[(M + H)^+ + 4]$, respectively.

The formation of target compounds 1-(4-(arylbenzylidene)-2-(arylthiazol-2-yl)hydrazine (**11a–r**) was also favored by their IR data. The disappearance of the bands for NH_2 and C=S near 3300 and 1080 cm^{-1} and the appearance of a band for C–S–C near 660 cm^{-1} evidenced the product. The compound **11b** (Fig. 4, Supplementary data) displayed stretching vibrations for −NH at 3290 cm^{-1}, 3088 cm^{-1}(Ar–H), 2933 and 2879 cm^{-1} (–C–H), 1625 cm^{-1} (C=N), 1512 cm^{-1} (C=C) and 663 cm^{-1} (C–S–C), respectively. Further, the ^{1}H NMR spectrum of **11b** (Fig. 11, Supplementary data) evidenced a characteristic singlet at δ 7.37 ppm for

thiazolyl-H. Similarly, two more singlets at δ 5.19 and 8.01 ppm were due to –O–CH_2 and =C–H protons, respectively. Along with this, the spectrum showed a doublet of doublet for the proton of C_5 of the 2,4-Cl_2-C_7H_5 ring at δ 7.49 ppm ($J = 2.0$ and 8.4 Hz). A doublet was observed for C_3–H due to meta-coupling at δ 7.70 with $J = 2.0$ Hz. Two more doublets at δ 7.80 ppm ($J = 8.8$ Hz) and 7.60 ppm ($J = 8.8$ Hz), respectively, were observed for 4 protons which are ortho and meta to the carbon-bearing bromine atom. A multiplet was predicted for two benzylidine protons and one benzyloxy protons in the range δ 7.63–7.65 ppm. A doublet was exhibited by C_4–H and $C_{4'}$–H protons of the benzylidine moiety at δ 7.10 ppm with J value 8.8 Hz. In the ^{13}C NMR of **11b** (Fig. 28, Supplementary data), purging of a signal for C=S and the emergence of a peak for -C-S- justified the thiazol-2-yl-hydrazone formation. Further, a signal observed at δ 66.4 ppm was manifested for O-CH_2 carbon. The 2nd and 5th carbon atoms of the thiazole ring resonated at δ 168.4 ppm and 104.3 ppm, respectively. Peaks at δ 159.1 ppm and 149.2 ppm correspond to C_4 of benzylidine as well as C_4 of the thiazole ring. An exocyclic –C=N carbon was found at δ 141.4 ppm and C_1 of the 2,4-Cl_2-C_7H_5 ring was resonated at δ 133.8 ppm. All the remaining aromatic carbons resonated in the region at δ 133.6, 133.3, 131.5, 131.4, 128.9, 127.9, 127.6, 127.5, 120.5 and 115.2 ppm, correspondingly. Chemical shift values of ^{1}H and ^{13}C NMR observed for the remaining compounds are summarised in the "Experimental" section. The ESI–MS spectral data of the same has assisted in confirming the new product formation, which showed a $(M + 1)^+$ peak at the m/z value of 531.9, along with isotopic peaks at m/z values of 534.03, 536.14 and 537.9 for $[(M + 1)^+ + 2]$, $[(M + 1)^+ + 4]$ and $[(M + 1)^+ + 6]$, respectively, which is in accordance with its molecular formula weight.

Biological activity

Anti-inflammatory activity and structural activity relationship (SAR)

To facilitate the prospective anti-inflammatory agent, the synthesized novel molecules (**11a–r**) were screened for their in vivo activity by opting carrageenan-induced paw edema method [37] in rats and the results are systematized in Table 1. The results obtained are distinctly showing that the compounds are modest to comparable activity to that of the Diclofenac sodium (standard). The compounds **11a, 11b, 11c, 11d, 11e** and **11g** showed significant activity when compared to the standard (53.2%). The derivatives **11c, 11d** and **11e** exhibited modest activity in 3 h duration after administration of the test compound and they started to act on the inflammation from the first hour. The compound encompassed by the (4-(2,4-dichlorobenzyloxy)-3-methoxybenzylidene) part in combination with (4-bromophenyl)thiazol-2-yl)hydrazine (**11e**) evolved as a potent moiety among the series, with 56% inhibition in 3 h duration and 58% inhibition was observed during the 2nd hour. The compound containing (4-(2,4-dichlorobenzyloxy)-3-methoxybenzylidene) which is substituted with 4-methylphenyl on the thiazole unit also exhibited 54% activity after 2 h of administration and remained active even after the

Table 1 In vivo anti-inflammatory activity data of the title compounds **11a–r**

Compound	1 h		2 h		3 h	
	Swelling	% inhibition	Swelling	% Inhibition	Swelling	% Inhibition
Control	0.256 ± 0.03	–	0.385 ± 0.01	–	0.391 ± 0.02	–
Diclofenac sodium	**0.2 ± 0.01*** **	**21.9**	**0.191 ± 0.02*** **	**50.4**	**0.183 ± 0.02*** **	**53.2**
11a	**0.250 ± 0.04***	**2.3**	**0.200 ± 0.04*** **	**48.1**	**0.186 ± 0.04*** **	**52.5**
11b	**0.202 ± 0.05***	**78.9**	**0.202 ± 0.04****	**47.5**	**0.186 ± 0.02*** **	**52.5**
11c	**0.172 ± 0.02****	**32.8**	**0.210 ± 0.07****	**45.5**	**0.182 ± 0.04*** **	**53.5**
11d	**0.190 ± 0.02***	**25.8**	**0.176 ± 0.04*** **	**54.3**	**0.180 ± 0.025*** **	**54.0**
11e	**0.180 ± 0.025***	**29.7**	**0.160 ± 0.036*** **	**58.4**	**0.172 ± 0.033*** **	**56.0**
11f	0.260 ± 0.014*	1.56	0.313 ± 0.066*	18.7	0.280 ± 0.068*	28.4
11g	**0.187 ± .033***	**27.0**	**0.187 ± .02*** **	**34.4**	**0.187 ± 0.04*** **	**52.2**
11h	0.270 ± 0.076*	5.5	0.322 ± 0.040*	16.4	0.243 ± 0.060*	37.9
11i	0.287 ± 0.042*	12.1	0.320 ± 0.07*	16.9	0.256 ± 0.047*	34.5
11j	0.280 ± 0.068*	9.4	0.317 ± 0.060*	17.7	0.293 ± 0.07*	25.1
11k	0.293 ± 0.088*	14.5	0.323 ± 0.079*	16.1	0.300 ± 0.036*	23.3
11l	0.263 ± 0.047*	2.7	0.330 ± 0.061**	14.3	0.283 ± 0.03*	27.6
11m	**0.196 ± 0.02***	**23.4**	**0.222 ± 0.02****	**42.3**	**0.232 ± 0.02*** **	**53.2**
11n	0.214 ± 0.01*	16.4	0.317 ± 0.01*	17.7	0.343 ± 0.02*	12.3
11o	0.238 ± 0.02*	7.0	0.333 ± 0.02*	13.5	0.380 ± 0.02*	2.8
11p	0.263 ± 0.02*	2.7	0.230 ± 0.02**	40.3	0.233 ± 0.03**	40.4
11q	0.188 ± 0.02*	26.6	0.322 ± 0.02*	16.4	0.360 ± 0.03*	7.9
11r	0.228 ± 0.02*	10.9	0.315 ± 0.02*	18.2	0.381 ± 0.03*	2.6

The data are presented as mean ± SEM, $n = 6$. Statistical analysis were performed using one-way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test

Levels of significance: *$P = 0.05$; **$P < 0.01$; $P <$ ***0.001 compared to control group. F value is 2.36

[a]Bold font indicates the significant anti-inflammatory activity of the compounds and standard drug

3rd hour. However, the compound with -2,4-Cl_2-C_6H_3 substitution on thiazol-2-yl-hydrazone is inactive. Compounds **11a, 11b, 11c** and **11g** displayed average 53% inhibition, remained effective up to the 3rd hour and the compound **11a** starts acting after the first hour of an injection. In the series, the compound bearing the (4-(2-fluorobenzyloxy)-3-methoxybenzylidene) moiety which is amalgamated with the (4-methylphenyl-thiazol-2-yl)hydrazine part is less active during the first hour, and activity increased consequently with time. In view of the result, (4-(2-fluorobenzyloxy)-3-methoxybenzylidene) derivatives **11h** and **11i** appeared to be moderately active with 38 and 35% inhibition, respectively. Molecules with the 5-bromothiophene part were found to be less active (23–28%) compared to the benzyloxy derivatives. Similarly, piperidine- and morpholine-bearing molecules were also having less activity except for **11m** and **11p**, which is having the 4-methlphenyl component at the thiazole portion, increasing the activity (53 and 40% inhibition, correspondingly). However, piperidine and morpholine part did not play any vital role in increasing the inhibitory action. It is clear that the molecules with benzyloxybenzylidine were found to be the most active compounds in the series. Moreover, 2,4-dichlorobenzyloxy nucleus substituted with 3-methoxybenzylidine snippet displayed significant activity (Fig. 2).

The results of the anti-inflammatory screening highlighted the facts about the relationship between the structure and the potency of the molecules (**11a–r**), which could assist us to select the propitious pharmacophore in this series. Interestingly, the results reflected the dependence of the molecular efficacy on the structural variations, which means that variations in the structure have altered the potency of the molecule. Fascinatingly, 4-(2,4-dichlorobenzyloxy)benzylidene derivatives produced effective anti-inflammatory activity. Further, substitution of the methoxy group on the 3rd position of the phenyl ring of the 4-(2,4-dichlorobenzyloxy)benzylidene enhanced the activity. However, the same with the 2,4-dichlorophenyl substituent on the thiazol-2-yl moiety substantially reduced the potency to 28.4%. However, replacement of 2,4-dichlorobenzyloxy by a 2-fluoro substituent decreased the anti-inflammatory property except for the compound with 4-methylphenylthiazol-2-yl, but the activity of 4-bromophenylthiazol-2-yl and 2,4-dichlorophenylthiazol-2-yl derivatives reduced to 37.9 and 34.5%, respectively. Notably, when the benzyloxy group was replaced by the 5-bromothiophene nucleus, a substantial decrease in the activity of all derivatives was observed. Further, replacement of the benzyloxy group by the 1-phenylpiperidine and 4-phenylmorpholine moieties significantly reduced the anti-inflammatory activity, but the derivatives with the 4-methylphenylthiazol-2-ly substituent did not reduce the activity.

In conclusion, benzyloxy-bezylidene was proved to be the key moiety to display the activity, which becomes further improved upon substitution with the –OMe group at the 3rd position of the benzylidene unit of the benzyloxy-benzylidine. Additionally, keen observation on the activity profile shows that the 4-Me substitution on the aryl moiety of thiazol-2-yl is also an essential facture to exhibit the activity.

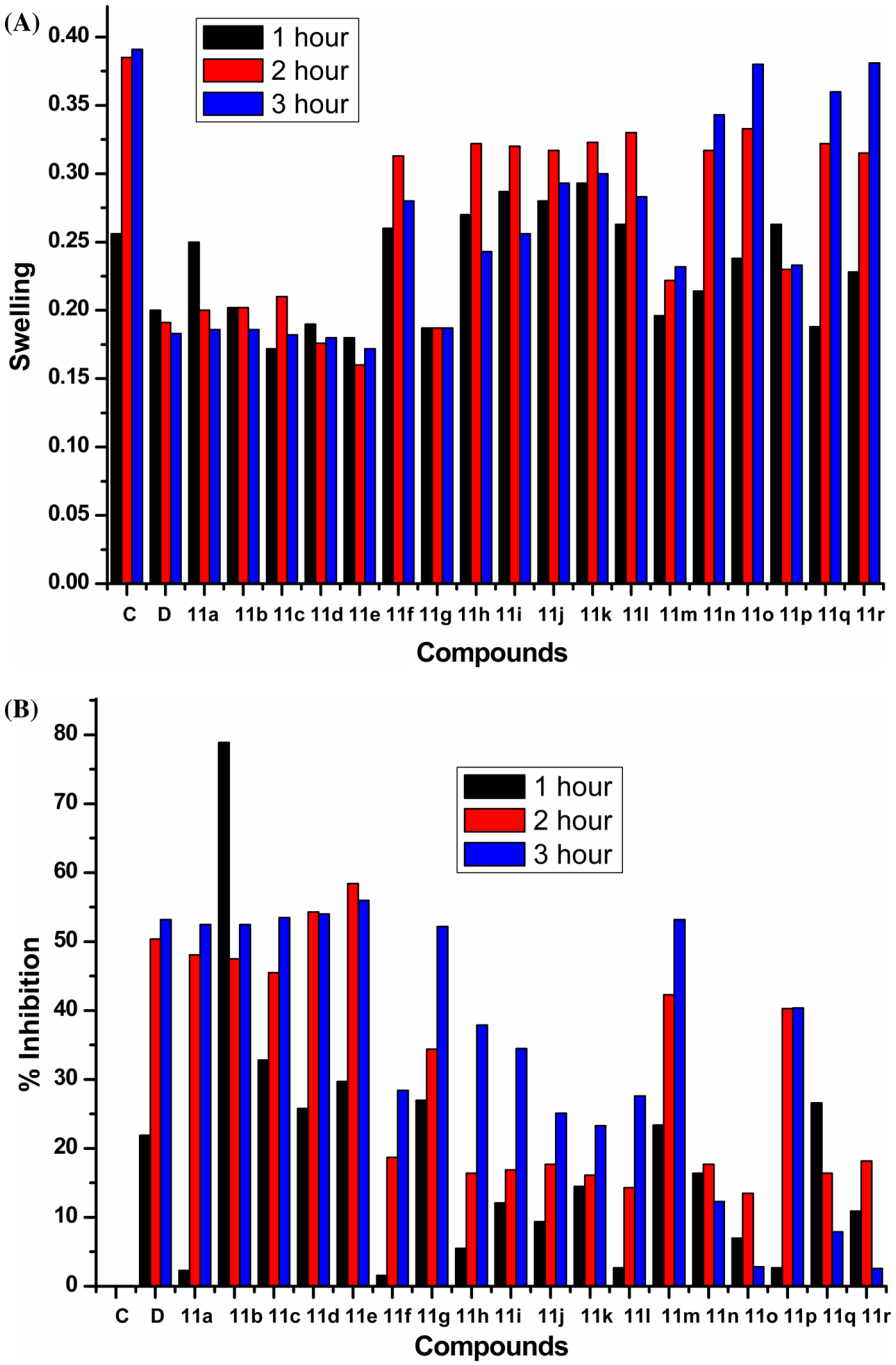


Fig. 2 **A** Effect of derivatives **11a–r** on swelling. **B** Percentage inhibition of inflammation using novel derivatives **11a–r**

Cytotoxicity

The compounds which are potent anti-inflammatory agents, **11d** and **11e**, were evaluated for their cytotoxicity against DLA (Dalton's lymphoma ascites) tumor cells at various concentrations (10, 20, 50, 100 and 200 μg/mL) using the trypan blue dye method. The cytotoxic effect of the tested compounds on the cell line is represented in Table 2. Both the tested compounds exhibited lower cytotoxicity on the tumor cells. Interestingly, the results reflect that the novel compounds **11d** and **11e** are nontoxic to the cells and showed only 30% of cell death at the highest concentration used (200 μg/mL).

Anti-oxidant property

The novel compounds bearing the benzyloxy moiety which showed significant anti-inflammatory activity were investigated for their anti-oxidant property by the DPPH scavenging method according to the Ref. [45]. IC_{50} and Imax values of the test compounds are represented in Table 3. From the results, it is noticeable that the compounds are less active when compared with the standard ascorbic acid, but compounds **11g, 11h** and **11c** showed moderate activity. However, the maximum inhibition of 90.5% was observed for compound **11c**, and the compounds **11g** and **11e** exhibited 78 and 76% inhibition, respectively. Interestingly, the derivatives which possess fluoro-substituents on the benzyloxy part were found to have better free radical scavenging activity when compared with the derivatives having chloro-

Table 2 Cytotoxicity of potent compounds **11d** and **11e** against the DAL tumor cell line

Compound	% Cell death				
	200 (μg/mL)	100 (μg/mL)	50 (μg/mL)	20 (μg/mL)	10 (μg/mL)
11d	26	16	5	2	0
11e	30	18	8	6	0

Table 3 Anti-oxidant activity (IC_{50}) and Imax values of the active compounds **11a–i**

Compounds	IC_{50} (μg/mL)	Imax (%)
11a	309.33	69.4
11b	273.75	74.9
11c	175.93	90.5
11d	267.52	69.6
11e	237.04	76.4
11f	209.77	65.4
11g	110.00	78.1
11h	146.96	65.3
11i	206.75	75.4
Ascorbic acid	18.68	–

IC_{50} concentration at which 50% inhibition was observed; *Imax* maximum % inhibition

substitution. Further, the structural adaptation on the thiazole unit may enhance the anti-oxidant activity.

Antibacterial activity

Eighteen molecules of the series were investigated for their in vitro antibacterial activity using the agar well diffusion method [41]. The zone of inhibition was determined at a concentration of 75 μg/mL against *Staphylococcus aureus* and *Bacillus subtilis* (Gram-positive) and *Pseudomonas aeruginosa* and *Escherichia coli* (Gram-negative) bacterial strains using Ciprofloxacin and Streptomycin as standards. Inhibitions obtained for the test compounds are tabulated in the Table 4. From the result, it is clear that almost all the compounds showed moderate activity against the tested bacterial strains. The compounds **11a, 11d, 11e** and **11r** showed modest activity against the Gram-negative as well as the Gram-positive bacteria. Benzyloxy derivatives as well as 5-bromothiphene derivatives showed nearly similar activity. Except for **11r**, all the other molecules with piperidine and morpholine were less potent when compared to benzyloxy derivatives.

Table 4 Zone of inhibition of target molecules **11a–r** in mm

Compounds	*Pseudomonas aeruginosa*	*Escherichia coli*	*Bacillus subtilis*	*Staphylococcus aureus*
11a	11.10	11.21	11.53	11.44
11b	9.57	08.31	14.16	15.12
11c	9.06	12.15	15.35	9.41
11d	13.07	19.71	14.61	9.46
11e	13.23	11.14	12.09	11.09
11f	13.20	10.24	8.22	11.15
11g	10.27	9.24	9.31	12.11
11h	9.85	11.76	13.72	11.50
11i	10.56	11.55	15.41	13.36
11j	11.12	11.04	12.63	9.24
11k	12.16	12.14	14.42	12.25
11l	10.36	12.24	13.25	10.16
11m	10.33	9.37	10.34	7.56
11n	9.43	10.47	11.57	7.68
11o	10.34	11.57	12.78	8.47
11p	11.58	9.46	10.74	12.45
11q	10.30	10.16	13.45	8.87
11r	13.43	10.81	19.50	9.75
Ciprofloxacin	22.20	21.10	20.54	23.31
Streptomycin	24.18	23.14	24.04	23.21

Mean of triplicate values

In silico molecular docking

An in silico molecular docking analysis has been undertaken to investigate the significant binding interactions of the target molecules with the binding pockets of the COX-2 protein [PDB ID: 3LN1], which is the principal requirement for producing the biological effect. The docking results obtained from the binding interaction of the molecules (**11a–r**) with the protein are set out in Table 5. The cDock energy of the compounds varied from − 48.1 to 12.8 kcal/mol.

In this series, most of the compounds (**11a, 11b, 11f, 11i, 11j, 11k, 11l** and **11r**) were found to bind actively with the Arg-120 of the COX-2 enzyme, which is the imperative amino acid among COX isoenzymes. The importance of Arg-120 is highly augmented for their involvement in increasing the specificity of NSAIDs among COX enzymes [46]. According to the literature on bovine COX, to have improved COX inhibitory activity there should be an interaction between Arg-120 and the fatty acids of the substrate via the guanidinium group. A ω-end of Arg-120 is situated in a hydrophobic area having a Ser-530 mantle in a scenario such that carbon-13 of the substrate has been positioned below Tyr-385 to have an appropriate COX reaction, and the Tyr-355 with phenolic side chain is sited close to the mouth of the conduit facing Arg-120 [47]. Additionally, the biochemical study of a complex of arachidonic acid (AA) and eicosapentaenoic acid (EPA) with COX-2 demonstrated that the flexibility of the Leu-531 side chain is the responsible feature in influencing the binding mode of AA and EPA with the protein, which in turn enhances the volume of the COX-2 channel for oxygenation of the wide array of fatty acids and esters. This analysis also demonstrated the importance of the interaction of the Agr-120 side chain with AA and EPA at the carboxylate end of the substrate in conquering the conformation flexibility. This scrutiny also confirmed that the side chain of Arg-120 has a conformational disparity in its arrangement around the substrates in COX-1 in contrast to COX-2 [48].

Furthermore, the literature clearly evidences the significance of Arg-120 in catalyzing the COX reactions. In addition, Arg-120 has also played an active role in the inhibitory effect of many of the commercially available drugs such as the inhibitor flurbiprofen binds to COX-2 through ion–pair interaction to the residues of Arg-120 via the carboxylate moiety [49]. Further, discernment of the structure of a complex of Diclofenac with murine COX-2 revealed that the drug has bonded to the protein with the carboxylate unit in an overturned configuration which concluded the interaction of Tyr-385 and Ser-530 through the hydrogen bond, in turn expelling the salt bridge formation with Arg-120. The excavation also advocated the importance of Ser-530 interaction of nimesulide in the inhibitory process [50].

The mechanism of action of novel compounds which showed significant in vivo activity (**11a, 11b, 11c, 11d, 11f** and **11m**) was predicted via the molecular docking study. In compound **11a** (Fig. 23, supplementary data), an aryl ring exhibited pi–pi interaction and S of the thiazole ring and N–H of the molecule formed a hydrogen bond (2.37605 and 2.44656 Å) with Arg-120 and Val-523, respectively. The derivative **11b** (Fig. 24, supplementary data) interacted with Arg-120 by forming a hydrogen bond with S of thiazole ring and double-bonded *N* with a bond distance of 2.46517 and 2.42724 Å, correspondingly. Two aryl rings of the compound also

Table 5 Molecular docking results of the novel compounds **11a–r** with COX-2 (PDB ID: 3LN1) protein

Compound code	CDOCKER energy (kcal/mol)	CDOCKER interaction energy (kcal/mol)	H-bond distance in Å	Interacting amino acids	Interaction ligand–residue
11a	− 4.21374	26.3753	2.37605	ARG 120	S of thiazole
			2.44656	VAL 523	N–H
11b	12.7738	36.4737	2.46517	ARG 120	S of thiazole
			2.42724		Double bonded N
11c	− 19.5893	23.6402	2.41808	TYR 355	Double bonded N
			2.05831		S of thiazole
11d	2.87036	31.4404	2.49502	LYS 83	Attached to Cl
			1.84999	TYR 355	S of thiazole
11e	1.09524	33.3042	1.96858	TYR 355	S of thiazole
11f	− 21.7465	25.1627	2.29789	ARG 120	S of thiazole
			2.49686	PRO 86	N–H
11g	2.22404	34.4908	2.03297	LYS 83	Attached to F
			1.92125	TYR 355	N–H
11h	− 3.91538	30.0495	2.41242	TYR 115	Attached to F
			1.89936	TYR 355	N–H
11i	− 13.2181	27.9945	2.42952	ARG 120	S ofthiazole
			2.48748	TYR 385	Attached to Cl
11j	− 1.96314	15.0451	2.42748	ARG 120	Double bonded N
			2.34393	GLU 524	N–H
11k	1.52658	20.3549	2.48922	ARG 120	S of thiazole
			2.41513	ARG120	S of thiophene
			2.17657	GLU524	N–H

Table 5 continued

Compound code	CDOCKER energy (kcal/mol)	CDOCKER interaction energy (kcal/mol)	H-bond distance in Å	Interacting amino acids	Interaction ligand–residue
11l	− 10.8425	14.5072	2.44281	ARG 120	Attached to Cl
			1.93567	TYR 355	S of thiophene
			2.48943	GLU 524	N–H
11m	− 16.813	20.3269	2.44908	ARG 513	S of thiazole
11n	− 17.6561	21.6782	2.40761	ARG 513	S of thiazole
11o	− 45.2729	1.85082	1.95111	TYR 355	S of thiazole
11p	− 14.2887	18.2597	–	–	–
11q	− 18.69	17.2224	–	–	–
11r	− 48.1458	2.69942	2.42933	ARG 120	Attached to Cl
			2.21502	GLU 524	S of thiazole
			2.42708	GLU 524	Double bonded N

communicated with Arg-120 and Lys-83 through pi-stacking. Similarly, the two aryl ring and a thiazole ring of the compound **11c** (Fig. 25, supplementary data), appeared to form a pi interaction with Arg-120, Arg-513 and Lys-83 with binding energy − 19.58 kcal/mol. Double-bonded nitrogen and thiazole S formed the hydrogen bond with Tyr-355. However, in the compound **11d** (Fig. 26, supplementary data), S of thiazole and chlorine present in the second position of benzyloxy part formed the hydrogen bonds with Tyr-355 and Lys-83 respectively. In unison, the compound established pi bonding with Lys-83.

According to Duggan et al., substitution of Tyr-355 by a surrogate phenylalanine countermanded the inhibitory role of naproxen. It necessitates the participation of Tyr-355 in inhibiting the COX-2 enzyme by thiazole-based molecules [51]. Fascinatingly, two aryl rings of the molecule **11e** (Fig. 27, supplementary data) exhibited two pi interactions with Lys-83 and Arg-120. Ring S formed a hydrogen bond with Tyr-355 amino acid. Intriguingly, Loll et al. [52] explained that Tyr-355 and Arg-120 are placed along the mouth of the channel and the catalytic receptacle in the COX. The interaction between these two residues indicates the conformational flexibility of the synthesized molecule.

It is evident that the variation in the docking energy and in vivo results may be attributed to the lack of structural flexibility of novel molecules. However, molecular docking interactions obviously indicated that the novel thiazole molecules have successfully communicated with the active site of the target protein.

Conclusions

In the present work, much attention has been given to the development of novel, small COX-2 inhibitors with significant anti-inflammatory, antimicrobial and anti-oxidant activities. From the data, it is evident that the molecules **11a, 11b, 11c, 11d, 11e** and **11g** are found to be significant inhibitors for inflammatory disorders among the series. However, the compounds **11d** and **11e** emerged as the most potent among them. The in silico binding interaction of the novel compounds with the COX-2 protein (PDB: 3LN1) revealed the vital binding interaction of the ligand with Arg-120, Tyr-385, Tyr-355 and Ser-530 amino acids, a necessary factor to act as a significant COX-2 inhibitor. Results acquired from the antibacterial screening legitimized the role of novel compounds in curing the infection caused by the intruders during the inflammatory disorder. Anti-oxidant activity results depicted the moderate effectiveness of the molecules in scavenging the free radicals generated in the medium.

In conclusion, the benzyloxy benzyledine is the key moiety in producing the good anti-inflammatory, antimicrobial, and anti-oxidant properties of the new thiazol-2-yl-hydrazones. Along with this, 4-methylphenyl substituent on the thiazol-2-yl unit and 3-OMe on the benzyloxybenzylidene played a prominent role in enhancing the activity. Further, the linkers (H–N–N=C–) S of the thiazole and aryl rings were helpful in producing the effective binding interactions with the amino acids of the protein. However, there is further scope to modify the molecular

structure to enhance the microbiological and anti-oxidant properties by varying the substituent at the aryl part of the thiazol-2-yl moiety.

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Compliance with ethical standards

Conflict of interest All the authors declare that, they have no Conflict of Interest.

Ethical approval This article contains the studies performed on animals (mice). The appropriate care and use of animals were performed according to the international, national, and institutional guidelines. The study on mice was in accordance with the ethical standards of the institutional and national research committee. This study does not include any human participants. Ethical No. SCSCP/IAEC/08/2016-2017.

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