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Some Novel Chalcone Derivatives containing 5-Chloro Thiophene in a Base Structure: Synthesis, Characterization, *in silico* Study and Biological Evaluation

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A novel series of chalcone derivatives (**4a-o**) of N-(4-acetylphenyl)-5-chlorothiophene-2-carboxamide was synthesized by coupling it with different substituted aromatic aldehydes. Synthesized chalcones were characterized by IR, NMR and mass spectra. They were screened *in vitro* antibacterial activity against four bacterial cultures and antifungal activity against three fungal cultures by using broth-dilution method. The significant antibacterial activity with ≤ 50 µg/mL has been displayed by compounds **4b** and **4n** against *E. coli* (MTCC 443), compounds **4b**, **4e**, **4i**, **4n** and **4o** against *P. aeruginosa* (MTCC 1688), compounds **4c** and **4n** against *S. aureus* (MTCC 96). *In silico* investigation was also carried out to predict pharmacokinetic properties (ADME) of synthesized chalcone derivatives.

Keywords: Chalcones, Thiophene, Antibacterial activity, Antifungal activity, ADME.

INTRODUCTION

Pathogenic microorganisms such as bacteria, viruses, fungi, and parasites are the primary cause of infectious illness transmission. Antimicrobial therapy is the usual treatment for infectious disorders, which involves the use of drugs that either destroy or stop the growth of microorganisms without hurting the host. Antimicrobial resistance (AMR), which refers to the ability of microorganisms to resist the effects of antibiotics, is mostly produced by the improper and excessive use of antimicrobial drugs. Additionally, there are several interconnected and mutually dependent factors contributing to this phenomenon [1]. Launched in year 2015, the World Health Organization's Global Action Plan on antimicrobial resistance aims to optimize the use of antimicrobial drugs and attract investment in their research and development, a field that has experienced a halt in recent decades [2]. To combat AMR, it is therefore essential to find novel promising antimicrobial drugs [3].

Heterocyclic compounds are the most common structural units of presently available medicines and common targets for the drug discovery process [4,5]. A synthetic applicability and wide range of biological activity makes heterocyclic compounds a most promising in drug discovery. With documented wide ranging of effects including anticancer, antibacterial, antifungal, anti-inflammatory, analgesics, antihypertensive, antitumor, antioxidant and local anesthetics properties, thiophene and their derivatives are a highly interesting class of heterocycllic chemicals [6,7]. For example, suprofen (non-steroidal anti-inflammatory), articaine (anesthetic), cefoxitin (antimicrobial agent), penthiopyrad (fungicide), raltitrexed (anticancer), thiophenfurin (antitumor), tiamenidine (antihypertension), duloxetine (anti-anxiety), rivaroxaban (anticoagulant) are some of thiophene based marketed drugs (Fig. 1).

In addition, chalcones are a class of natural and synthetic compounds, of a great interest for a medicinal chemist because of having specific chemical structure consisting of two aromatic rings connected of three carbon α,β-unsaturated carbonyl system [8-10]. Chalcones are extensively dispersed throughout many plant sources. Their varied biological functions and their uses in a drug discovery have drawn a lot of attention [11-14]. Wide range of pharmacological properties, including anticancer [15-18], anti-inflammatory [19-22], antioxidant [23,24], antimicrobial [25-29], antiviral [30], antidiabetic activities [31], among others make chalcones valuable candidate for developing novel therapeutic agents. Numerous studies have focused on the synthesis, modification and structural optimization of chalcones to enhance their biological activity and selectivity [32]. For therapeutic applications, some drugs based on chalcones have received

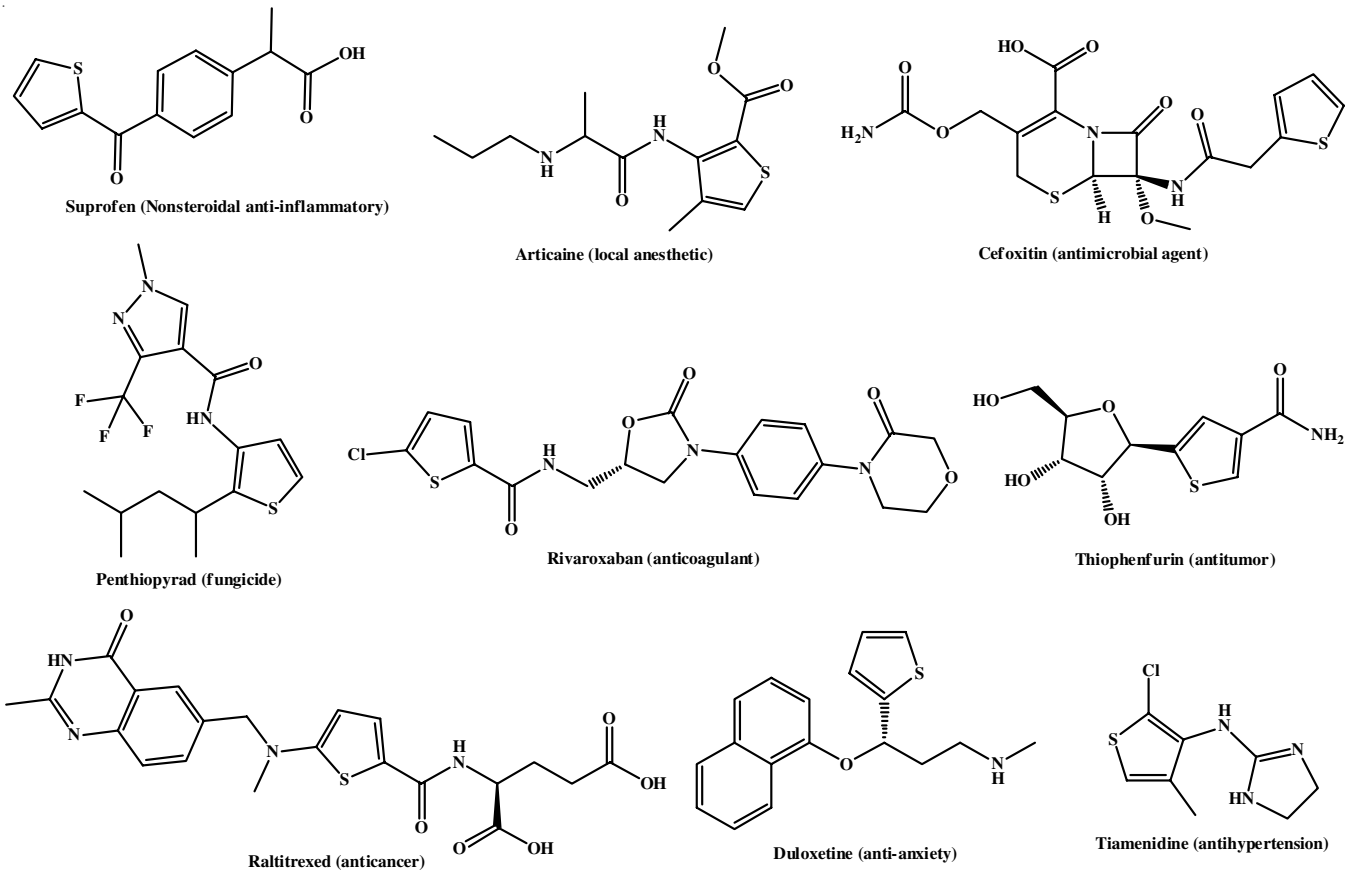


Fig. 1. Structure of some marketed medicines based on thiophene

approval, for instance, sofalcone was formerly used as an anti-ulcer and mucoprotective medication, hesperidin methyl-chalcone used as a vascular protective, whilst metochalcone was sold as choleretic/diuretics drgus (Fig. 2) [33,34].

In light of these informations and with the objective of developing effective antibacterial substances, the current study focuses on the synthesis of a range of innovative heterocyclic compounds (**4a-o**) that have thiophene and chalcone as their

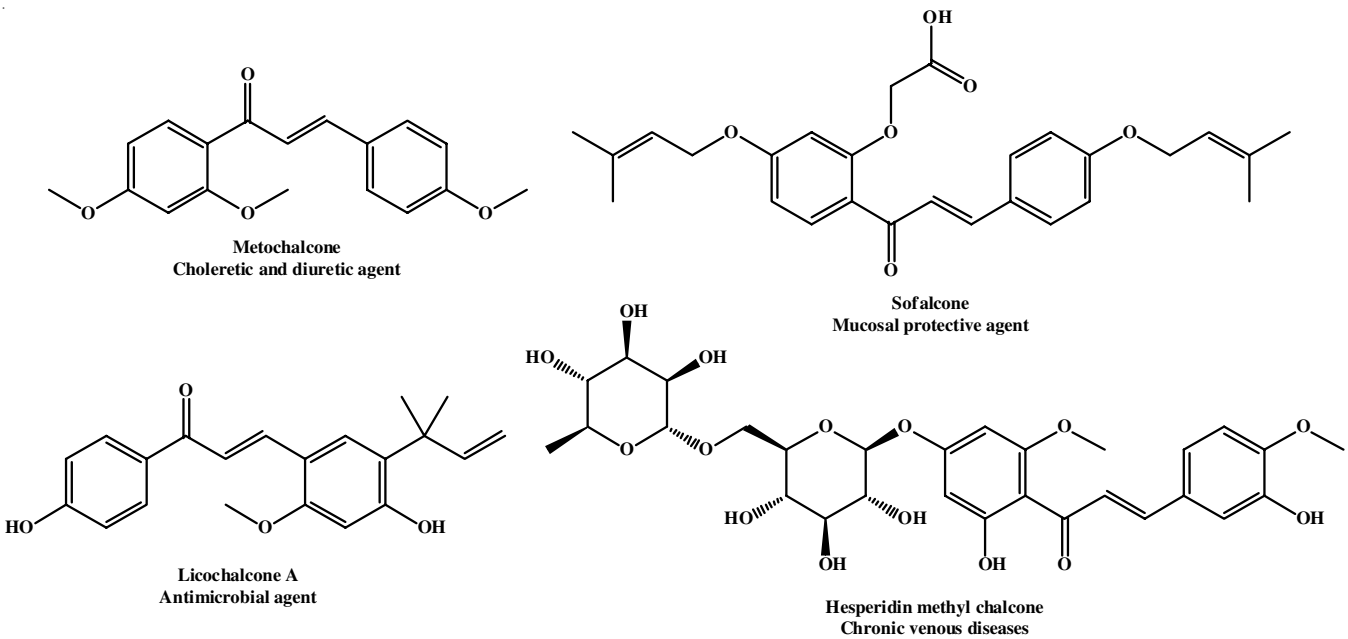


Fig. 2. Structure of some approved chalcones in the market

structural components. Additionally, the antibacterial activity of the synthesized compounds was assessed *in vitro* against four bacterial strains and their pharmacokinetic parameters (ADME) were predicted using *in silico* methods.

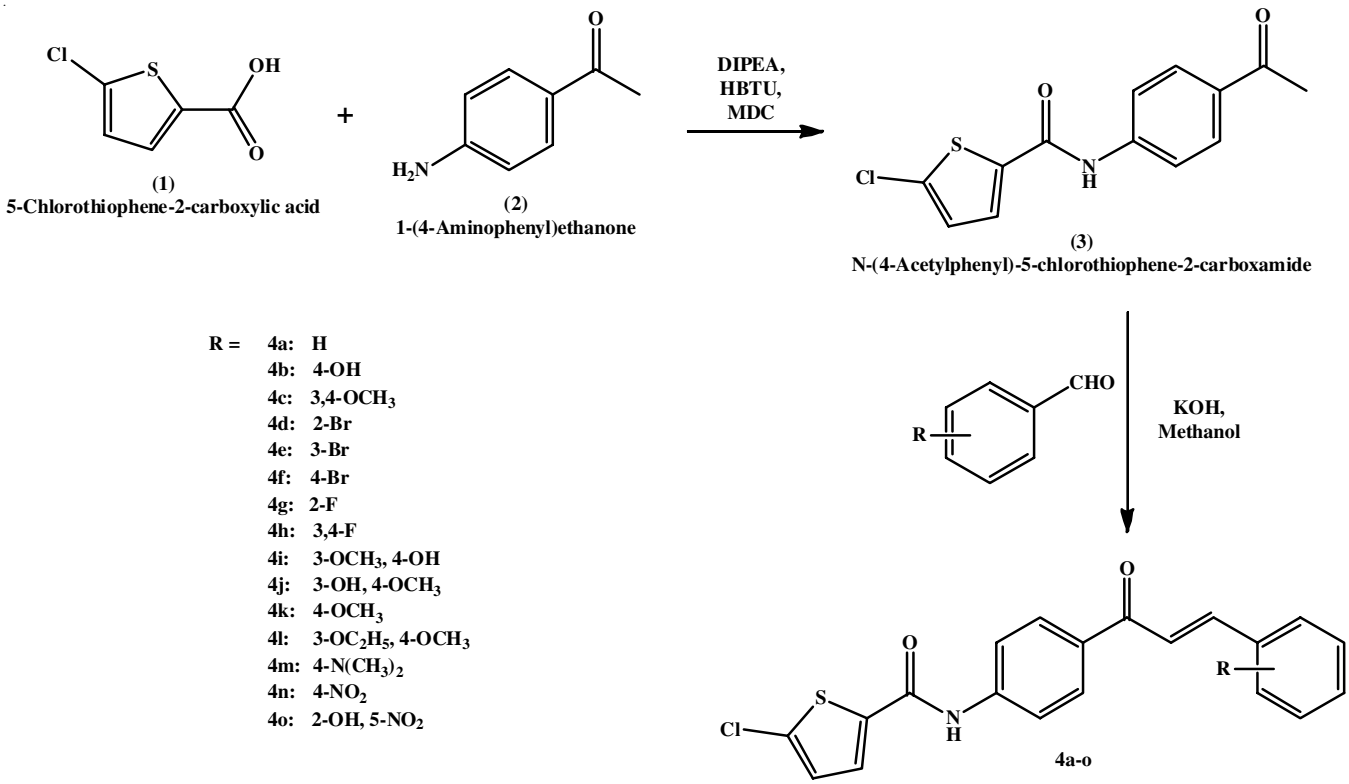
EXPERIMENTAL

Only laboratory grade reagents were used in the synthesis of the compounds and commercial solvents were used without further additional purification. All the melting points of the synthesized compounds were measured using the open capillary method and are uncorrected. Thin layer chromatography (TLC) was used to track the reaction's progress using Merck 60 F_{254} silica gel aluminum plates, while UV light (254 nm) was used to visualize the spots. Shimandzu-8400 FT-IR spectrometer was used to obtain FT-IR spectra, whereas Applied Biosystems-API 2000 LC/MS/MS was used to record mass spectra. A Bruker Advance Neo 400 MHz spectrometer was used to record ^{1}H & ^{13}C NMR spectra.

Synthesis of *N*-(4-acetylphenyl)-5-chlorothiophene-2-carboxamide (3): 1-(4-Aminophenyl)ethanone (*p*-amino acetophenone) (**2**) (0.012 mol) dissolved in DMF (16 mL) was added to diisopropyl ethylamine (DIPEA, (0.03 mol) and 5-chlorothiophene-2-carboxylic acid (**1**, 0.01 mol). Then hexafluorophosphate benzotriazole tetramethyluranium (HBTU) was added slowly and then refluxed for 6 h. TLC was used to verify the consumption of starting material by employing MDC: methanol (9.8:0.2) as mobile phase. Reaction mixture was poured in (50 mL) water and then methylene dichloride (MDC) (30 mL) was charged in the reaction mixture. Reaction mixture was filtered to remove salt. The MDC layer was washed by using 25 mL of water for 5 times and then concentrated. Methyl *tert*-butyl ether (MTBE , 16 mL) was added to the residue and it was agitated for 30 min was then filtered and dried the solid **3** in vacuum oven at 45-50 ºC. IR (KBr, ν_{max}, cm^{-1}): 3335 (N-H), 3059 (C-H), 3001 (C-H), 1658 (C=O), 1597 (C=O), 1529 (N-H), 1427 (C=C), 844 (C-Cl), 808 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.56 (s, 3H), 7.31 (d, *J* = 4.1 Hz, 1H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.99 (d, *J* = 9.0 Hz, 3H), 10.60 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 26.99, 39.38, 39.59, 39.80, 40.01, 40.21, 40.42, 40.63, 119.95, 128.90, 129.86, 130.28, 132.72, 135.06, 139.11, 143.30, 159.62, 197.08. Mass *m/z*: 280.2 (M+1)$^+$.

Synthesis of chalcone derivatives (4a-o): Compound **3** (0.01 mol) was taken in a round bottom flask containing 27 mL methanol and then added to the substituted benzaldehyde derivatives dissolved in 40% KOH solution. The reaction mixture was agitated at 60-65 ºC for 12-18 h (**Scheme-I**). Reaction completion was monitored by TLC using methanol:MDC (0.2:9.8). Solid was filtered and recrystallized by methanol.

5-Chloro-N-(4-cinnamoylphenyl)thiophene-2-carboxamide (4a): Yield: 38.02%; m.p.: 160-162 ºC, m.f.: $C_{20}H_{14}NO_2SCl$ (m.w.: 367.85). IR (KBr, ν_{max}, cm^{-1}): 3390 (N-H), 3091 (C-H), 3059 (C-H), 1656 (C=O), 1595 (C=O), 1512 (N-H), 1427 (C=C), 837 (C-Cl), 808 (C-H), 796 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.64 (s, 1H), 8.22 (d, *J* = 8.8 Hz, 2H), 8.01 (d, *J* = 2.2 Hz, 1H), 7.98 (d, *J* = 9.2 Hz, 1H), 7.96-7.88 (m, 4H), 7.75 (d, *J* = 15.6 Hz, 1H), 7.48 (s, 3H), 7.32 (d, *J* = 4.1 Hz, 1H).^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 188.07, 159.64, 143.98, 143.44, 139.14, 135.27, 135.11, 133.33, 131.01, 130.29, 129.39, 129.33, 128.89, 122.43, 120.06. Mass *m/z*: 368.3 (M+1)$^+$.



Scheme-I

5-Chloro-N-(4-(3-(4-hydroxyphenyl)acryloyl)phenyl)-thiophene-2-carboxamide (4b): Yield: 43.73%; m.p.: 210-212 ºC, m.f.: $C_{20}H_{14}NO_3SCl$ (m.w.: 383.85). IR (KBr, ν_{max}, cm^{-1}): 3390 (N-H), 3085 (C-H), 3048 (C-H), 1669 (C=O), 1592 (C=O), 1524 (N-H), 1428 (C=C), 1220 (C-O) 845 (C-Cl), 811 (C-H), 775 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.98 (s, 1H), 8.70 (s, 1H), 7.97 (d, *J* = 15.1 Hz, 1H), 7.82 (d, *J* = 7.5 Hz, 2H), 7.64 (dd, *J* = 7.4, 2.8 Hz, 3H), 7.41 (d, *J* = 15.1 Hz, 1H), 7.14 (d, *J* = 7.4 Hz, 2H), 6.98 (d, *J* = 7.5 Hz, 1H), 6.71 (d, *J* = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 190.98, 159.30, 156.58, 144.36, 143.89, 141.28, 135.96, 134.20, 132.86, 129.96, 129.98, 129.58, 129.56, 127.94, 127.62, 122.95, 121.94, 121.92, 116.37, 116.38. Mass *m/z*: 384.8 $(M+1)^+$.

5-Chloro-N-(4-(3-(3,4-dimethoxyphenyl)acryloyl)-phenyl)thiophene-2-carboxamide (4c): Yield: 52.30%; m.p.: 145-147 ºC, m.f.: $C_{22}H_{18}NO_4SCl$ (m.w.: 427.90). IR (KBr, ν_{max}, cm^{-1}): 3346 (N-H), 3091 (C-H), 2960 (C-H), 2933 (C-H), 1678 (C=O), 1581 (C=O), 1518 (N-H), 1427 (C=C), 823 (C-Cl), 802 (C-H), 729 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.62 (s, 1H), 8.22 (d, *J* = 8.6 Hz, 2H), 8.00 (s, 1H), 7.93 (d, *J* = 8.5 Hz, 2H), 7.87 (d, *J* = 15.5 Hz, 1H), 7.71 (d, *J* = 15.4 Hz, 1H), 7.56 (s, 1H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.32 (s, 1H), 7.03 (d, *J* = 8.3 Hz, 1H), 3.88 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 187.94, 159.63, 151.72, 149.53, 144.52, 143.23, 139.17, 135.07, 133.65, 130.26, 130.15, 128.86, 128.11, 124.39, 120.02, 119.94, 112.03, 111.22, 56.26, 56.08. Mass *m/z*: 428.3 $(M+1)^+$.

N-(4-(3-(2-Bromophenyl)acryloyl)phenyl)-5-chloro-thiophene-2-carboxamide (4d): Yield: 50.10%; m.p.: 150-152 ºC, m.f.: $C_{20}H_{13}NO_2SBrCl$ (m.w.: 446.74). IR (KBr, ν_{max}, cm^{-1}): 3342 (N-H), 3026 (C-H), 1658 (C=O), 1612 (C=O), 1526 (C=C), 1508 (N-H), 1423 (C=C), 838 (C-Cl), 796 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.35 (d, *J* = 15.1 Hz, 1H), 7.85 (d, *J* = 7.4 Hz, 2H), 7.64 (d, *J* = 7.4 Hz, 3H), 7.50 (d, *J* = 15.2 Hz, 1H), 7.39 (dd, *J* = 7.4, 1.4 Hz, 1H), 7.24-6.95 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 190.98, 156.58, 143.89, 143.31, 141.28, 137.10, 135.96, 134.20, 134.02, 132.86, 130.01, 129.58, 129.56, 129.17, 127.94, 127.74, 126.66, 123.18, 121.92, 121.94. Mass *m/z*: 447.7 $(M+1)^+$.

N-(4-(3-(3-Bromophenyl)acryloyl)phenyl)-5-chlorothiophene-2-carboxamide (4e): Yield: 75.14%; m.p.: 186-188 ºC, m.f.: $C_{20}H_{13}NO_2SBrCl$ (m.w.: 446.74). IR (KBr, ν_{max}, cm^{-1}): 3352 (N-H), 3086 (C-H), 1649 (C=O), 1600 (C=O), 1531 (C=C), 1518 (N-H), 1425 (C=C), 831 (C-Cl), 792 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.64 (s, 1H), 8.25 (dd, *J* = 5.1, 3.5 Hz, 3H), 8.07 (d, *J* = 15.6 Hz, 1H), 8.00 (d, *J* = 4.1 Hz, 1H), 7.94 (d, *J* = 8.8 Hz, 2H), 7.87 (d, *J* = 7.8 Hz, 1H), 7.68 (dd, *J* = 24.8, 11.8 Hz, 2H), 7.43 (t, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 4.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 187.87, 159.65, 143.59, 142.17, 139.13, 137.80, 135.13, 133.41, 133.13, 131.38, 131.22, 130.41, 130.31, 128.87, 128.73, 123.90, 122.90, 120.01. Mass *m/z*: 447.6 $(M+1)^+$.

***N*-(4-(3-(4-Bromophenyl)acryloyl)phenyl)-5-chloro-thiophene-2-carboxamide (4f):** Yield: 75.14%; m.p.: 198-200 ºC, m.f.: $C_{20}H_{13}NO_2SBrCl$ (m.w.: 446.74). IR (KBr, ν_{max}, cm^{-1}): 3362 (N-H), 3076 (C-H), 1662 (C=O), 1620 (C=O), 1531 (C=C), 1512 (N-H), 1418 (C=C), 827 (C-Cl), 792 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.98 (s, 1H), 7.99 (d, *J* = 15.1 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 2H), 7.64 (dd, *J* = 7.5, 3.1 Hz, 3H), 7.43 (dd, *J* = 29.5, 11.3 Hz, 3H), 7.18 (d, *J* = 7.4 Hz, 2H), 6.98 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 190.98, 156.58, 144.36, 143.89, 141.28, 135.96, 135.77, 134.20, 132.86, 132.27, 132.27, 129.58, 129.56, 129.54, 129.52, 127.94, 124.18, 122.95, 121.94, 121.92. Mass *m/z*: 447.6 $(M+1)^+$.

5-Chloro-N-(4-(3-(2-fluorophenyl)acryloyl)phenyl)-thiophene-2-carboxamide (4g): Yield: 58.00%; m.p.: 174-176 ºC, m.f.: $C_{20}H_{13}NO_2SClF$ (m.w.: 385.84). IR (KBr, ν_{max}, cm^{-1}): 3348 (N-H), 3066 (C-H), 1652 (C=O), 1614 (C=O), 1527 (C=C), 1509 (N-H), 1420 (C=C), 839 (C-Cl), 780 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.34 (d, *J* = 15.1 Hz, 1H), 7.84 (d, *J* = 7.6 Hz, 2H), 7.65 (dd, *J* = 7.5, 1.2 Hz, 3H), 7.43 (d, *J* = 15.1 Hz, 1H), 7.32-7.23 (m, 1H), 7.20-7.09 (m, 1H), 7.04-6.90 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 190.98, 158.55, 156.58, 143.89, 141.28, 140.76, 135.96, 134.20, 132.86, 130.59, 130.12, 129.58, 129.56, 127.94, 126.05, 125.44, 122.53, 121.94, 121.92, 116.76. Mass *m/z*: 386.5 $(M+1)^+$.

5-Chloro-*N*-(4-(3-(3,4-difluorophenyl)acryloyl)-phenyl)thiophene-2-carboxamide (4h): Yield: 41.56%; m.p.: 169-171 ºC, m.f.: $C_{20}H_{12}NO_2SF_2Cl$ (m.w.: 403.83). IR (KBr, ν_{max}, cm^{-1}): 3356 (N-H), 3082 (C-H), 1661 (C=O), 1612 (C=O), 1528 (C=C), 1505 (N-H), 1432 (C=C), 834 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.23 (d, *J* = 15.1 Hz, 1H), 7.83 (d, *J* = 7.6 Hz, 2H), 7.65 (d, *J* = 7.5 Hz, 3H), 7.55 (d, *J* = 15.1 Hz, 1H), 7.13-7.02 (m, 2H), 6.96 (ddd, *J* = 12.7, 8.7, 6.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 190.98, 156.58, 152.92, 148.15, 144.33, 143.89, 141.28, 135.96, 134.25, 134.20, 132.86, 129.58, 129.57, 127.94, 126.22, 123.24, 121.94, 121.92, 116.84, 114.56. Mass *m/z*: 404.5 $(M+1)^+$.

5-Chloro-*N*-(4-(3-(4-hydroxy-3-methoxyphenyl)acryl-oyl)phenyl)thiophene-2-carboxamide (4i): Yield: 40.56%; m.p.: 188-190 ºC, m.f.: $C_{21}H_{16}NO_4SCl$ (m.w.: 413.87). IR (KBr, ν_{max}, cm^{-1}): 3316 (N-H), 3055 (C-H), 1643 (C=O), 1608 (C=O), 1551 (C=C), 1516 (N-H), 1423 (C=C), 840 (C-Cl), 786 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.80 (s, 3H). 6.68 (t, *J* = 8.8 Hz, 1H), 6.84-6.74 (m, 2H), 6.98 (d, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 15.1 Hz, 1H), 7.64 (dd, *J* = 7.4, 2.4 Hz, 3H), 7.82 (d, *J* = 7.5 Hz, 2H), 7.96 (d, *J* = 15.1 Hz, 1H), 8.56 (s, 1H), 9.99 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 190.98, 156.58, 149.57, 148.50, 144.33, 143.89, 141.28, 135.96, 134.20, 132.86, 129.58, 129.56, 127.94, 127.58, 123.24, 123.18, 121.94, 121.92, 115.81, 111.78, 56.79. Mass *m/z*: 414.5 $(M+1)^+$.

5-Chloro-*N*-(4-(3-(3-hydroxy-4-methoxyphenyl)-acryloyl)phenyl)thiophene-2-carboxamide (4j): Yield: 40.55%; m.p.: 125-127 ºC, m.f.: $C_{21}H_{16}NO_4SCl$ (m.w.: 413.87). IR (KBr, ν_{max}, cm^{-1}): 3371 (N-H), 3079 (C-H), 1667 (C=O), 1616 (C=O), 1527 (C=C), 1506 (N-H), 1416 (C=C), 845 (C-Cl), 760 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.00 (s, 2H), 8.09 (s, 3H), 7.97 (d, *J* = 15.1 Hz, 3H), 7.81 (d, *J* = 7.4 Hz, 6H), 7.64 (dd, *J* = 7.5, 2.2 Hz, 9H), 7.40 (d, *J* = 15.1 Hz, 3H), 6.99 (d, *J* = 7.5 Hz, 3H), 6.86-6.78 (m, 6H), 6.70-6.63 (m, 3H), 3.80 (s, 9H), 2.00 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 190.98, 156.58, 150.33, 146.89, 144.33, 143.89, 141.28, 135.96, 134.20, 132.86, 129.75, 129.58, 129.56, 127.94,

123.24, 121.94, 121.92, 120.86, 113.52, 112.85, 56.79. Mass *m/z*: 414.6 $(M+1)^+$.

5-Chloro-*N*-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)-thiophene-2-carboxamide (4k): Yield: 70.31%; m.p.: 164-166 ºC, m.f.: $C_{21}H_{16}NO_3SCl$ (m.w.: 397.87). IR (KBr, ν_{max}, cm^{-1}): 3372 (N-H), 3064 (C-H), 1658 (C=O), 1608 (C=O), 1524 (C=C), 1509 (N-H), 1436 (C=C), 824 (C-Cl), 785 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.19 (d, *J* = 15.1 Hz, 1H), 7.85 (d, *J* = 7.4 Hz, 2H), 7.74 (d, *J* = 7.5 Hz, 1H), 7.66 (d, *J* = 7.4 Hz, 2H), 7.53 (d, *J* = 15.1 Hz, 1H), 7.28 (d, *J* = 7.4 Hz, 2H), 6.97 (d, *J* = 7.5 Hz, 1H), 6.84 (d, *J* = 7.4 Hz, 2H), 3.78 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 190.98, 160.84, 156.58, 144.36, 143.89, 141.28, 135.96, 134.20, 132.86, 129.58, 129.56, 129.38, 129.38, 128.73, 127.94, 122.95, 121.94, 121.92, 114.57, 114.55, 56.04. Mass *m/z*: 398.7 $(M+1)^+$.

5-Chloro-*N*-(4-(3-(3-ethoxy-4-methoxyphenyl)acryl-oyl)phenyl)thiophene-2-carboxamide (4l): Yield: 91.27%; m.p.: 170-172 ºC, m.f.: $C_{23}H_{20}NO_4SCl$ (m.w.: 441.93). IR (KBr, ν_{max}, cm^{-1}): 3385 (N-H), 3048 (C-H), 1686 (C=O), 1606 (C=O), 1529 (C=C), 1511 (N-H), 1421 (C=C), 824 (C-Cl), 754 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.01 (d, *J* = 15.1 Hz, 1H), 7.82 (d, *J* = 7.6 Hz, 2H), 7.65 (dd, *J* = 7.6, 5.0 Hz, 3H), 7.39 (d, *J* = 15.1 Hz, 1H), 6.95 (ddd, *J* = 12.7, 8.9, 4.4 Hz, 3H), 6.80 (d, *J* = 7.4 Hz, 1H), 4.03 (q, *J* = 6.0 Hz, 2H), 3.79 (s, 3H), 1.39 (t, *J* = 6.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 190.98, 156.58, 152.17, 149.60, 144.33, 143.89, 141.28, 135.96, 134.20, 132.86, 130.25, 129.58, 129.56, 127.94, 123.24, 122.13, 121.94, 121.92, 114.29, 114.20, 64.52, 56.79, 13.83. Mass *m/z*: 442.7 $(M+1)^+$.

5-Chloro-*N*-(4-(3-(4-(dimethylamino)phenyl)acryloyl)-phenyl)thiophene-2-carboxamide (4m): Yield: 65.35%; m.p.: 162-164 ºC, m.f.: $C_{22}H_{19}N_2O_2SCl$ (m.w.: 410.92). IR (KBr, ν_{max}, cm^{-1}): 3355 (N-H), 3018 (C-H), 1645 (C=O), 1604 (C=O), 1516 (C=C), 1502 (N-H), 1427 (C=C), 841 (C-Cl), 756 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.97 (d, *J* = 15.2 Hz, 1H), 7.82 (d, *J* = 7.5 Hz, 2H), 7.65 (dd, *J* = 7.5, 5.5 Hz, 3H), 7.37 (d, *J* = 15.1 Hz, 1H), 7.12 (d, *J* = 7.4 Hz, 2H), 6.99 (d, *J* = 7.5 Hz, 1H), 6.55 (d, *J* = 7.4 Hz, 2H), 2.88 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 190.98, 156.58, 151.12, 144.36, 143.89, 141.28, 135.96, 134.20, 132.86, 129.93, 129.91, 129.58, 129.56, 127.94, 123.80, 122.95, 121.94, 121.92, 112.73, 112.71, 41.92, 41.91. Mass *m/z*: 411.6 $(M+1)^+$.

5-Chloro-*N*-(4-(3-(4-nitrophenyl)acryloyl)phenyl)-thiophene-2-carboxamide (4n): Yield: 81.30%; m.p.: 176-178 ºC, m.f.: $C_{20}H_{13}N_2O_4SCl$ (m.w.: 412.85). IR (KBr, ν_{max}, cm^{-1}): 3375 (N-H), 3060 (C-H), 1678 (C=O), 1605 (C=O), 1532 (C=C), 1504 (N-H), 1416 (C=C), 1323 (C-N), 834 (C-Cl), 754 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.18-8.09 (m, 3H), 7.83 (d, *J* = 7.5 Hz, 2H), 7.74 (d, *J* = 15.1 Hz, 1H), 7.65 (dd, *J* = 7.5, 5.4 Hz, 3H), 7.56 (d, *J* = 7.4 Hz, 2H), 6.99 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 190.98, 156.58, 147.81, 144.36, 143.89, 142.40, 141.28, 135.96, 134.20, 132.86, 129.58, 129.56, 129.14, 129.12, 127.94, 124.46, 124.45, 122.95, 121.93, 121.92. Mass *m/z*: 413.6 $(M+1)^+$.

5-Chloro-*N*-(4-(3-(2-hydroxy-5-nitrophenyl)acryl-oyl)phenyl)thiophene-2-carboxamide (4o): Yield: 39.14%; m.p.: 202-204 ºC, m.f.: $C_{20}H_{13}N_2O_5SCl$ (m.w.: 428.85). IR (KBr, ν_{max}, cm^{-1}): 3383 (N-H), 3046 (C-H), 1654 (C=O), 1613 (C=O), 1521 (C=C), 1503 (N-H), 1424 (C=C), 1328 (C-N), 826 (C-Cl), 724 (C-H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.54 (s, 1H), 10.00-7.97 (m, 3H), 10.00-7.89 (m, 4H), 10.00-7.79 (m, 6H), 10.00-7.71 (m, 7H), 10.00-7.02 (m, 10H), 10.00-1.69 (m, 12H), 10.00-2.02 (m, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 190.98, 163.70, 156.58, 143.89, 141.28, 140.25, 139.39, 135.96, 134.20, 132.86, 129.58, 129.57, 127.94, 126.22, 123.30, 123.24, 121.93, 121.92, 120.11, 118.23. Mass *m/z*: 427.6 $(M+1)^+$.

Antimicrobial activity: The non-automated broth-dilution method was utilized to estimate the minimal inhibition concentration (MIC) of all the synthesized chalcone derivatives of N-(4-acetylphenyl)-5-chlorothiophene-2-carboxamide (**4a-o**). Using this technique, the quantity of antimicrobial drugs required to stop the growth of a particular microbe can be determined quantitatively [35]. *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 442) are the bacterial strains obtained from Institute of Microbial Technology, Chandigarh, India used for screening the antibacterial activity. In order to obtain the proper inoculum size of 10^8 CFU mL^{-1} in each well using the microdilution method, a standardized inoculum for each bacterial strain was generated by comparing the turbidity. To prepare a stock solution, each synthesized molecule was diluted in DMSO to a concentration of 2000 µg/mL. The synthesized drug concentrations of 1000 µg/mL, 500 µg/mL and 250 µg/mL were taken for primary screening. To achieve the necessary concentration in each test well of a microtiter plate, each dilution was spread on microtiter plates with two-fold strength (2X) Mueller Hinton broth (MH broth). After that, the microtiter plates were incubated at 37 ºC. A standard inoculum size of 50 µL of bacterial suspension was added to each test and growth control well for inoculation. The active synthetic medications identified by this first screening were examined in a second round of dilution tests against microbe. The substances recognized as active in the first screening were diluted in a similar way to yield 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL and 12.5 µg/mL. Before being inoculated, the antibiotic-free control tube was immediately subculture by equally spreading a loopful of medium over a fourth of a plate that was suitable for the test organism's development. The plate was then incubated at 37 ºC for 24 h. Turbidity was used to identify the bacterial growth after the incubation period [36,37].

***In silico* study (ADME analysis):** To anticipate the physico-chemical property, an *in silico* investigation of the synthesized compounds (**4a-o**) was conducted in this study. Swiss ADME web tool used to assist with lipophilicity, water solubility, pharmacokinetics, drug similarity and medicinal chemistry. The synthesized compounds' structures were sketched in Chem-Draw Ultra 12.0 and translated into SMILES format for prediction.

RESULTS AND DISCUSSION

The synthetic route for preparing novel chalcone derivatives of N-(4-acetylphenyl)-5-chlorothiophene-2-carboxamide has

been shown in **Scheme-I**. In first step, 5-chlorothiophene-2-carboxylic acid (**1**) and 1-(4-aminophenyl)ethanone (**2**) were coupled by simple acid amine coupling reaction resulted in the formation of N-(4-acetylphenyl)-5-chlorothiophene-2-carboxamide (**3**). Then compound **3** was condensed with substituted aromatic aldehydes by refluxing it in presence of base results in desired chalcone derivatives of N-(4-acetylphenyl)-5-chlorothiophene-2-carboxamide (**4a-o**).

Biological evolution

Antibacterial activities: Results of antibacterial activity of compounds (**4a-o**) are shown in Table-1. Against Gram-negative, *E. coli* (MTCC 443), compounds **4b** and **4n** displayed good activity. Both compounds exhibit slightly more activity than chloramphenicol and ciprofloxacin, with a minimum inhibitory concentration (MIC) of 12.5 µg/mL. Against Gram-negative, *P. aeruginosa* (MTCC 1688), compounds **4b**, **4e**, **4i**, **4n** and **4o** displayed good activity by having MIC 6.25 µg/mL, 25 µg/mL, 12.5 µg/mL, 25 µg/mL and 50 µg/mL, respectively. Chloramphenicol is similar to a compound **4o**, while compounds **4e** and **4n**, it has a higher level than chloramphenicol and is equivalent to ciprofloxacin. However, in the case of compounds **4b** and **4i**, it has a higher level than both chloramphenicol and ciprofloxacin. Against Gram-positive *S. aureus* (MTCC 96), compounds **4c** and **4n** displayed good activity by having MIC 25 and 50 µg/mL, respectively. For compound **4c**, it is more than chloramphenicol and ciprofloxacin combined, but for compound **4n**, it is equivalent with those two standard drugs. None of the compounds showed any activity comparable to or higher than that of any conventional drug against Gram-positive *S. pyogenus* (MTCC 442).

TABLE-1
ANTIBACTERIAL ACTIVITY OF SYNTHESIZED DERIVATIVES (**4a-o**)

Compound	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pyogenus*
3	50	62.5	100	100
4a	125	100	100	250
4b	12.5	6.25	100	125
4c	100	62.5	25	125
4d	250	250	125	250
4e	62.5	25	100	250
4f	125	125	250	100
4g	125	125	125	62.5
4h	250	250	250	100
4i	125	12.5	100	62.5
4j	250	250	125	100
4k	125	250	500	500
4l	250	100	250	500
4m	100	62.5	500	250
4n	12.5	25	50	100
4o	125	50	62.5	125
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Norfloxacin	10	10	10	10

***In silico* analysis:** The results of the WLOGP vs. TPSA function, as shown in Fig. 3 and Table-2, were predicted for the blood brain barrier (BBB) penetration and human gastrointestinal absorption (HIA) using the BOILED-egg model developed by the SwissADME online base tool. In the graphical representation white region shows high possibility of passive absorption by gastrointestinal track and yellow region shows high possibility of brain penetration. Blue coloured dots are predicted as an active effluxed by P-gp (PGP^+) and red dots are not substrate of P-gp (PGP^-).

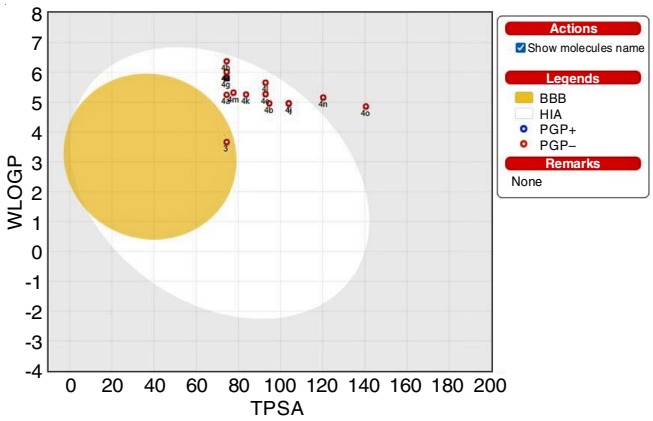


Fig. 3. Brain or Intestinal EstimateD permeation method (BOILED-egg) predictive graphical representation of synthesized derivatives (**4a-o**)

TABLE-2
PHYSICO-CHEMICAL PROPERTY FOR BOILED-EGG METHOD

Compound	m.w.	WLOGP	TPSA (Å)	PGP subtract	GI absorption	BBB permeant
3	279.74	3.67	74.41	No	High	Yes
4a	367.85	5.25	74.41	No	High	No
4b	383.85	4.96	94.64	No	High	No
4c	427.90	5.27	92.87	No	High	No
4d	446.74	6.01	74.41	No	High	No
4f	446.74	6.01	74.41	No	High	No
4g	385.84	5.81	74.41	No	High	No
4h	403.83	6.37	74.41	No	High	No
4i	413.87	4.96	103.87	No	High	No
4j	413.87	4.96	103.87	No	High	No
4k	397.87	5.26	83.64	No	High	No
4l	441.93	5.66	92.87	No	High	No
4m	410.92	5.32	77.65	No	High	No
4n	412.85	5.16	120.23	No	Low	No
4o	428.85	4.86	140.46	No	Low	No

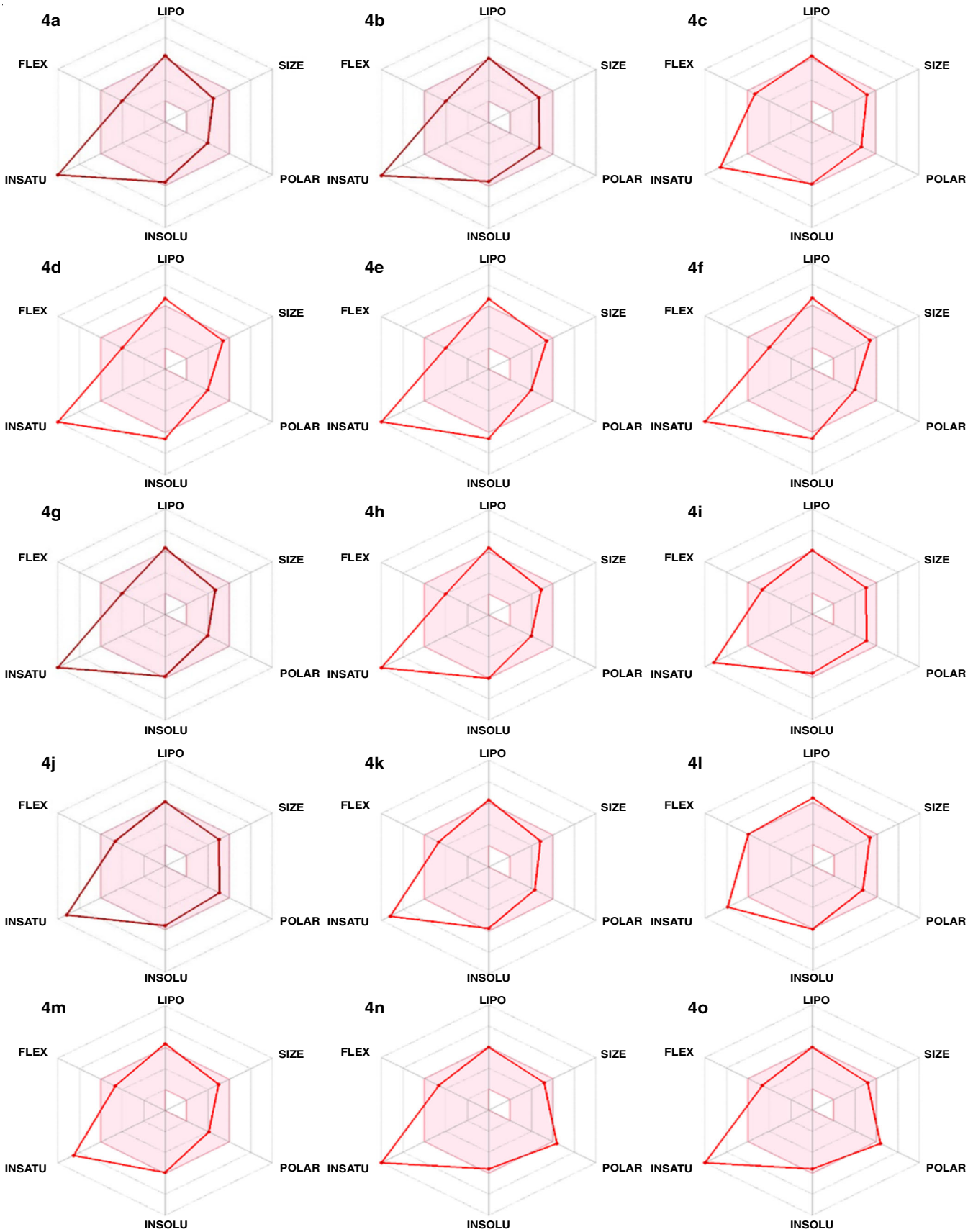


Fig. 4. Bioavailability radar, A glance at a drug-likeness of synthesized derivatives (**4a-o**) [LIPO: Lipophilicity as a XLOGP3 between -0.7 and 5.0. Size: molecular size between 150-500 g/mol. POLAR: Polarity as TPSA between 20-130 Å^2. INSOLU: Insolubility (0 < Log S (ESOL) < 6). INSATU: in saturation as fraction of carbons in the sp^3 hybridization < 0.25. FLEX: Flexibility as no rotatable bonds should be more then 9]

TABLE-3
PHYSICO-CHEMICAL PROPERTY FOR BIOAVAILABILITY RADAR

Compound	XLOGP3	m.w.	TPSA (Å)	ESOL Log S	Fraction Csp3	#Rotatable bonds
3	3.42	279.74	74.41	-3.92	0.08	4
4a	5.50	367.85	74.41	-5.69	0	6
4b	5.15	383.85	94.64	-5.55	0	6
4c	5.44	427.90	92.87	-5.83	0.09	8
4d	6.19	446.74	74.41	-6.60	0	6
4e	6.19	446.74	74.41	-6.60	0	6
4f	6.19	446.74	74.41	-6.60	0	6
4g	5.60	385.84	74.41	-5.85	0	6
4h	5.70	403.83	74.41	-6.00	0	6
4i	5.12	413.87	103.87	-5.62	0.05	7
4j	5.12	413.87	103.87	-5.62	0.05	7
4k	5.47	397.87	83.64	-5.76	0.05	7
4l	5.81	441.93	92.87	-6.07	0.13	9
4m	5.62	410.92	77.65	-5.92	0.09	7
4n	5.33	412.85	120.23	-5.74	0	7
4o	4.97	428.85	140.46	-5.60	0	7

Pharmacokinetics studies: Drug likeness of all the synthesized derivatives (**4a-o**) were evaluated by SwissADME web base tool by predicting the physico-chemical properties like lipophilicity, size, polarity, solubility, flexibility and saturation and the results are shown in Table-3. The bioavailability radar pink area (Fig. 4) represent the optimum range for each property.

Conclusion

A series of *N*-(4-acetylphenyl)-5-chlorothiophene-2-carboxamide chalcone derivatives (**4a-o**) has been successfully synthesized with a good to average yield and characterized by spectral analysis. *In vitro* antibacterial study revealed that all the compounds show moderate to good antibacterial activity. Among all the synthesized compounds, compounds **4n** inhibit both Gram-positive and Gram-negative bacteria probable due to presence of -NO_2 group. From their physico-chemical studies all the compounds show good gastrointestinal absorption and no blood brain barrier permeability and show drug-likeness characteristic. All these studies revealed that potency of these compounds as a future antibiotic drug.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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