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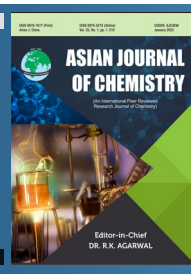

Coumarin Imidazolone Hybrid as Inhibitor of Topoisomerase Type II and Hsp90 and as Potent Antimicrobials

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In present study, coumarin imidazolone hybrids were synthesized on the basis of the molecular docking analysis of the compounds (**11a-h**) by using PyRx 0.8 software. The molecules docking studies were performed against the targets Hsp90 and human type IIA DNA topoisomerase and compared the same with standard drugs fluconazole and doxorubicin. Among all the compounds **11d** (-12.7 kcal/mol) and **11f** (-12.4 kcal/mol) showed good binding affinity towards human Hsp90 and **11d** (-11.5 kcal/mol) and **11e** (-11.4 kcal/mol) showed good binding affinity against human type IIA DNA topoisomerase. Based on the positive results a series of eight novel titled compounds (1-(4-(4-(arylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl)phenyl)ethylidene)-2-oxo-2*H*-chromene-3-carbohydrazides (**11a-h**) have been prepared by the method of condensation of 1-(4-acetylphenyl)-4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones (**5a-h**) with 2-oxo-2*H*-chromene-3-carbohydrazide (**10**). Characterization of the synthesized compounds were conducted by FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectral studies and screened for their antioxidant, anticancer and antimicrobial activities. The compounds were analyzed for physico-chemical and pharmacokinetic properties of all the compounds showed good to moderate properties.

Keywords: Imidazolones, Coumarins, Human Hsp90, Human Type IIA DNA topoisomerases, Anticancer activity.

INTRODUCTION

Despite the fact that there are number of anticancer drugs available in the market, but several limitations of these treatments have urged researchers to continue seeking for new anticancer drugs. However, it is generally known that heterocycles provide fascinating skeletons for the molecules that interact with targets and obstruct the biological processes involved in the spread of cancer. Since it is simple to develop these ring structures with several substituent's, they can span a large amount of chemical space, which makes them important starting places for the design and discovery of anticancer drug [1].

For many years, invasive microbial infections are a major issue all over the world, prevalent in individuals with suppressed immune systems. The present growth in antimicrobial medicine research is a result of the urgent demand for innovative antimicrobial treatments to treat these lethal invasive illnesses. Antimicrobial resistance has become more prevalent in this century, necessitating the development of novel antimicrobial agents that are more effective, selective and safe for use in clinical settings. Numerous biological activities, including as antibacterial and antifungal characteristics, are discovered in heterocycles with an azole ring structure [2,3].

Reactive oxygen species (ROS), which are capable of damaging DNA, have been linked to coronary heart disease, carcinogenesis and a variety of other age-related health issues. Antioxidants work by removing or stopping the production of ROS, which may shield against the development of free radicals [4]. It is also reported that when the microbes are under the stress conditions, free radicals will be generated which is the initiation step of mutagenesis because of which antimicrobials resistance will be developed, therefore by free radical scavenging activity the resistance developed by the microbes can be combated. As free radicals are also involved in the pathophysiology of cancer, if any of the agent is possessing both antioxidant and anticancer activity it can show synergistic effects [5].

Many imidazoles are particularly effective against organisms that are multidrug resistant and imidazole derivatives have

a range of pharmacological features, including anticancer action [6,7]. Because coumarins have fused benzene and pyrone ring structures, biological study is needed to determine their potential therapeutic value. It has a strong anticancer potential and few negative effects. Coumarins have a remarkable capacity to control a wide variety of cellular pathways, which may be investigated for specific anticancer efficacy [8]. As a consequence, the coumarin-imidazole hybrid molecule offers a potential model for the creation of new anticancer prospects that may be successful against both drug-sensitive or drug-resistant cancers.

Heat shock proteins (Hsps) are known as stress induced proteins (Hsp90s) are the protecting DNA damage and apoptosis induced by several stress conditions including oxidative stress [9]. They expressed in responses to stress in cell, in normal conditions they are not expressed in cells. Type IIA DNA topoisomerase involved in several roles such as chromatin condensation, chrom-osome segregation, modulation of topological state of DNA, management of DNA structure, that controlled by hydrolysis, ATP binding and release of inorganic phosphate and ADP [10]. A crucial target for cancer treatment is human DNA topoisomerase II [11]. Studies have revealed that targeting Hsp90 and Type IIA DNA topoisomerase with chemical inhibitors is found to be effective antibiotics, antimicrobial agents. Hsp90 has been targeted in recent years for the treatment of cancer, for a neuroprotective approach to treating Alzheimer's illness by dissolving protein clumps and as antifungal drugs to overcome the resistance generated by bacteria [12].

In current study, we have focused to produce compounds with dual action (anticancer and antifungal) by inhibiting Hsp90s and Type IIA DNA topoisomerase, which has maximum binding affinity with the targeted protein under investigation, with improved solubility because currently available Hsp90 and Type IIA DNA topoisomerase inhibitors, which are in clinical trials and obtained from either synthetic or natural products have solubility and toxicity problems. The promising therapeutic perspectives of both the heterocycles inspired us towards the development of novel and potent Schiff base linked coumarin, imidazole derivatives. Hence, in this work, the synthesis, characterization, docking, drug likeness, physico-chemical properties and biological evaluation of (1-(4-(4-(arylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl)phenyl)ethylidene)-2-oxo-2*H*-chromene-3-carbohydrazides (**11a-h**) were carried out.

EXPERIMENTAL

Merck and Sigma-Aldrich, USA used were all of the AR grade in this work. By using silica gel F_{254} (Merck) for TLC and visualizing the results using UV light, the purity of the chemicals was examined. OptiMelt automated melting point system was determined melting points in uncorrected capillaries that are open. TLC utilizing silica gel F_{254} (Merck) and perception by UV-light utilized to establish the purity of the chemicals and compounds. Utilizing column chromatography on silica gel, the synthesized compounds were purified (60-120 mesh). The spectroscopic data were collected using the following instruments: FT-IR spectrophotometer SHIMADZU-435, ^{13}C NMR ($CDCl_3$, Inova 75 MHz) ^1H NMR ($CDCl_3$, Avance 300 MHz), OPTIZEN3220 UV-Visible spectrophotometer, with chemical shifts (δ) provides in ppm in relation with TMS as an internal standard. The molecular docking studies were carried out utilizing PyRx 0.8 software.

General methods: General process for the synthesizing benzoyl glycine (**1**) and 4-(arylidene)-2-phenyloxazol-5(4*H*)-ones (**3a-h**). Benzoyl glycine (**1**) was synthesized by reacting glycine in NaOH solution with benzoyl chloride,which upon the condensation with different aryl aldehydes (**2**) leads to the formation of 4-(arylidene)-2-phenyloxazol-5(4*H*)-ones (**3a-h**) [13].

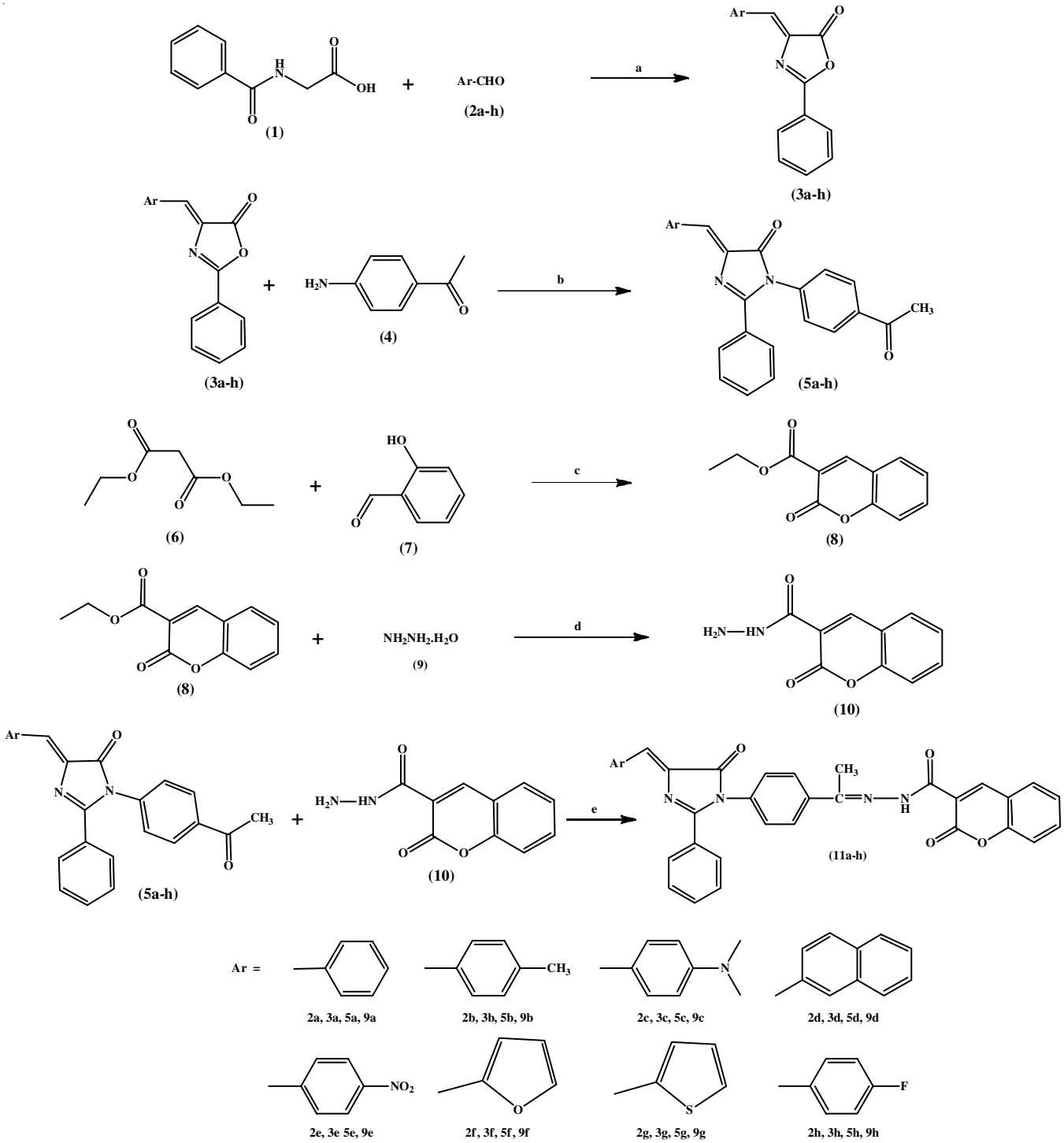
Synthesis of 1-(4-acetylphenyl)-4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones (5a-h): DMF-$POCl_3$ reagent was synthesized by adding slowly anhydrous DMF (5 mL) to $POCl_3$ (1.96 mmol) with continuous stirring at 0 ºC for upto 45 min. The oxazolinones (**3a-h**), (0.01 M), *p*-aminoacetophenone (**4**), (0.01 M) were added and then refluxed on water bath for 2 h. The TLC monitored the progress of the reaction. In order to settle the generated product, by cooling the reaction mixture, by placing it in ice-cold water and left alone a short period of time. After cooling, the solid was filtered, collected, washed with cold water and methanol, and then dried in vacuum. Chromatography and recrystallization processes were used to purify the products (**Scheme-I**).

1-(4-Acetylphenyl)-4-benzylidene-2-phenyl-1*H*-imidazol-5(4*H*)-one (5a): Yellow colour powder; yield: 80%; m.p.: 210-212 ºC; IR (KBr, ν_{max}, cm^{-1}): 3020 (Ar-H *str.*), 2673 (aliphatic-CH *str.*), 1717 (C=O), 1680 (C=C *str.*), 1023 (C-N *str.*); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 8.00 (d, 2H, Ar-H, *J* = 8.11 Hz), 7.47 (m, 7H, Ar-H & Ar-CH=), 7.15 (d, 2H, Ar-H, *J* = 7.75 Hz), 6.90 (m, 4*H*, Ar-H), 2.50 (s, 3H, -CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 198.24, 169.23, 161.46, 153.68, 143.83, 131.07, 129.91, 127.96, 127.56, 126.16, 126.08, 125.53, 123.20, 121.11, 117.38, 116.93, 110.08, 26.48; ESI-MS (*m/z*): 367 $(M+1)^+$.

1-(4-Acetylphenyl)-4-(4-methylbenzylidene)-2-phenyl-1*H*-imidazol-5(4*H*)-one (5b): Yellowish red colour powder; yield: 76%; m.p.: 212-214 ºC; IR (KBr, ν_{max}, cm^{-1}): 3130.78 (Ar-H *str.*), 2971.00 (aliphatic-CH *str.*), 1672 (C=O), 1096.79 (C-N *str.*); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 8.05 (m, 4*H*, Ar-H), 7.60 (m, 6H, Ar-H), 7.30 (m, 4*H*, Ar-H & Ar-CH=), 2.65 (s, 3H, Ar-CH_3), 2.15 (s, 3H, -$COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 197.69, 169.18, 164.71, 143.45, 138.97, 131.84, 129.69, 128.32, 127.59, 129.90, 126.51, 125.15, 120.97, 11.64, 114.78, 110.19, 97.24, 26.51, 21.84l; ESI-MS (*m/z*): 381 $(M+1)^+$.

1-(4-Acetylphenyl)-4-(4-(dimethylamino)benzylidene)-2-phenyl-1*H*-imidazol-5(4*H*)-one (5c): Brick red colour powder; yield: 80%; m.p.: 224-226 ºC; IR (KBr, ν_{max}, cm^{-1}): 3134 (Ar-H *str.*), 2896 (aliphatic-CH *str.*), 1753 (C=O), 1065 (C-N *str.*); ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.75 (m, 6H, Ar-H), 7.25 (s, 1H, Ar-CH=), 2.20 (s, 3H, -CH_3), 3.20 (s, 6H, -$N(CH_3)_2$), 2.20 (s, 3H, -CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 198.51, 169.67, 166.08, 153.93, 142.00, 132.21, 128.58, 127.38, 125.79, 120.58, 116.76, 109.52, 106.40, 41.36, 27.51; ESI-MS (*m/z*): 410 $(M+1)^+$.

1-(4-Acetylphenyl)-4-(naphthalen-2-ylmethylene)-2-phenyl-1*H*-imidazol-5(4*H*)-one (5d): Yellowish red; yield:



Scheme-I: Synthesis of coumarin-imidazolone derivatives (a) anhy. sodium acetate, acetic anhydride, reflux for 2 h; (b) DMF/$POCl_3$, 2 h reflux; (c) piperidine, 6 h reflux; (d) methanol, 7 h reflux; (e) ethanolic NaOH, 8 h reflux

78%; m.p.: 217 ºC; IR (KBr, ν_{max}, cm^{-1}): 3164 (Ar-H *str.*), 2906 (aliphatic-CH *str.*), 1718 (C=O), 1095 (C-N *str.*); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 8.00 (d, 2H, Ar-H, *J* = 8.66 Hz), 7.70 (t, 3H, Ar-H), 7.40 (m, 8H, Ar-H & Ar-CH=), 7.05 (m, 2H, Ar-H), 6.85 (d, 2H, Ar-H), 2.45 (s, 3H, -$COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 198.42, 169.69, 164.54, 153.83, 144.46, 139.05, 131.23, 128.35, 128.29, 127.53, 125.82, 120.73, 120.64, 116.92, 109.17, 109.00, 26.29; ESI-MS (*m/z*): 417 $(M+1)^+$.

1-(4-Acetylphenyl)-4-(4-nitrobenzylidene)-2-phenyl-1*H*-imidazol-5(4*H*)-one (5e): Yellow; yield: 80%; IR (KBr, ν_{max}, cm^{-1}): 3098 (Ar-H *str.*), 2764 (aliphatic-CH *str.*), 1643 (C=O), 1123 (C-N *str.*); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.98 (m, 5H, Ar-H), 7.65 (m, 4*H*, Ar-H), 7.35 (m, 6H, Ar-H), 2.51 (s, 3H, -$COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 198.51, 167.19, 166.88, 142.00, 136.83, 131.76, 128.58, 127.38, 125.79, 120,52, 116.76, 109.52, 106.40, 27.51; ESI-MS (*m/z*): 412 $(M+1)^+$.

1-(4-Acetylphenyl)-4-(furan-2-ylmethylene)-2-phenyl-1*H*-imidazol-5(4*H*)-one (5f): Brown; yield: 68%; IR (KBr, ν_{max}, cm^{-1}): 3034 (Ar-H *str.*), 2996 (aliphatic-CH *str.*), 1750 (C=O), 1055 (C-N *str.*); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 8.12 (d, 2H, Ar-H, *J* = 8.35 Hz), 7.95 (m, 4*H*, Ar-H), 7.70 (s, 1H, Ar-CH=), 7.50 (m, 6H, Ar-H), 2.50 (s, 3H, -COCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 198.36, 169.76, 166.08, 165.36, 153.93, 142.00, 136.19, 135.83, 130.53, 128.58, 127.38, 125.79, 120.52, 116.76, 109.52, 106.40, 27.15; ESI-MS (*m/z*): 385 $(M+1)^+$.

1-(4-Acetylphenyl)-2-phenyl-4-(thiophen-2-ylmethylene)-1*H*-imidazol-5(4*H*)-one (5g): Brick red; yield: 70%; IR (KBr, ν_{max}, cm^{-1}): 3016 (Ar-H *str.*), 2784 (aliphatic-CH *str.*), 1790 (C=O), 1082 (C-N *str.*); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.90 (d, 2H, Ar-H, *J* = 8.76 Hz), 7.50 (m, 5H, Ar-H), 7.25 (s, 1H, Ar- CH=), 7.10 (m, 5H, Ar-H), 2.25 (s, 3H, -COCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 198.51, 167.19, 166.08, 142.00, 136.83, 131.76, 128.58, 127.38, 125.79, 120.52, 116.76, 109.52, 106.40, 27.51; ESI-MS (*m/z*): 373 $(M+1)^+$.

1-(4-Acetylphenyl)-4-(4-fluorobenzylidene)-2-phenyl-1*H*-imidazol-5(4*H*)-one (5h): Brown; yield: 69%; IR (KBr, ν_{max}, cm^{-1}): 3098 (Ar-H *str.*), 2934 (aliphatic-CH *str.*), 1776 (C=O), 1134 (C-N *str.*); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.85 (d, 2H, Ar-H *J* = 8.45 Hz), 7.45(m, 5H, Ar-H), 7.31(s, 1H, Ar-CH=), 7.15 (m, 3H, Ar-H), 6.90 (m, 3H, Ar-H), 2.42(s, 3H, -COCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 198.36, 169.76, 166.08, 165.36, 153.93, 142.00, 136.19, 135.83, 130.53, 128.58, 127.38, 125.79, 120.52, 116.76, 109.52, 106.40, 27.51; ESI-MS (*m/z*): 385 $(M+1)^+$.

Synthesis of ethyl-2-oxo-2*H*-chromene-3-carboxylate (8): To a mixture of salicylaldehyde (**6**) (0.05 M, 6 mL), diethylmalonate (**7**) (0.05 M, 7 mL) in (15 mL) methanol and 0.1 mL piperidine were added and then refluxed for 6 h on a waterbath. Through TLC monitoring, the completion of the reaction was confirmed. Ice-cold water was added then, a pale yellow colour solid was separated, filtered and dried after being collected. The obtained crude product was purified using column chromatography and recrystallized from ethanol, m.p. 81-82 ºC; yield: 82%.

Synthesis of 2-oxo-2*H*-chromene-3-carbohydrazide (10): Ethyl-2-oxo-2*H*-chromene-3-carboxylate (**8**) (0.07 M, 1.9 g) and hydrazine hydrate (**9**) (0.02 M) dissolved in absolute methanol was taken in RBF and refluxed for 7 h. Through TLC monitoring, the reaction's completion was checked. After cooling, the reaction mixture was placed in to ice water, then the separated solid was collected by filtration, purified and dried.

2-Oxo-2*H*-chromene-3-carbohydrazide (10): Cream; yield: 82%; m.p.: 204 ºC; m.f.: $C_{11}H_{10}N_2O_2$; IR (KBr, ν_{max}, cm^{-1}): 3404.90 (N-H *str.*), 3040.06 (Ar-H *str.*), 2974.61 (aliphatic C-H *str.*), 1704.58 (pyrone C=O), 1623.32 (C=N), 1253.90 (C-O *str.*), 1175.71 (C-N *str.*); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 9.91 (s, 1H,-CONH), 8.51 (s, 1H, coumarin lactone), 7.62 (t, 2H, Ar-H, *J* = 7.85 Hz), 7.25 (d, 2H, Ar-H, *J* = 7.66 Hz), 4.25 (s, 2H, -NH$_2$ hydrazide); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 164.08, 159.49, 153.83, 129.40, 128.29, 127.53, 125.82, 120.73, 116.92, 109.00; ESI-MS (*m/z*): 205 $(M+1)^+$.

Synthesis of (4-(4-(arylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl)phenyl)ethylidene)-2-oxo-2*H*-chromene-3-carbohydrazides (11a-h): A mixture of 1-(4-acetylphenyl)-4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones (**5a-h**) and 2-oxo-2*H*-chromene-3-carbohydrazide (**10**) (0.01 M), NaOH traces were taken in 25 mL of ethanol and refluxed. The separated product was filtered, then methanol was added after a cold water wash and then finally dried in vacuum.

(1-(4-(4-Benzylidene-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1yl)phenyl)ethylidene)-2-oxo-2*H*-chromene-3-carbohydrazide (11a): Yellow powder; yield: 77%; m.p.: 270-272 ºC; IR (KBr, ν_{max}, cm^{-1}): 3394 (N-H *str.*), 3064 (Ar-H *str.*), 2925 (aliphatic-CH *str.*), 1758 (C=O), 1694 (pyrone C=O *str.*), 1639 (C=N *str.*), 1575 (C=C *str.*), 1228 (C-O *str.*), 1033 (C-N *str.*); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 12.20 (s, 1H, -CONH), 8.50 (s, 1H, coumarin lactone), 7.82 (m, 3H, Ar-H, *J* = 8.62 Hz), 7.50 (m, 9H, Ar-H & Ar-CH=), 7.10 (m, 5H, Ar-H), 6.53 (s, 1H, Ar-H), 1.81 (s, 3H, -CH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 169.49, 164.83, 164.43, 141.41, 135.21, 132.21, 129.49, 128.38, 127.62, 125.76, 120.76, 116.92, 110.34, 16.54; ESI-MS (*m/z*): 551 $(M-1)^+$.

(1-(4-(4-Methylbenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazolyl)phenyl)ethylidene)-2-oxo-2*H*-chromene-3-carbohydrazide (11b): Yellow colour powder; yield: 77%; m.p.: 250-252 ºC; IR (KBr, ν_{max}, cm^{-1}): 3335 (N-H *str.*), 3030 (Ar-H *str.*), 2922 (aliphatic-CH *str.*), 1753 (C=O), 1715 (pyrone C=O *str.*), 1613 (C=N *str.*), 1515 (C=C *str.*), 1199 (C-O *str.*), 1040 (C-N *str.*); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 12.20 (s, 1H, -CONH), 8.59 (s, 1H, coumarin lactone), 7.72 (m, 5H, Ar-H & Ar-CH=), 7.20 (m, 4*H*, Ar-H), 6.90 (m, 8H, Ar-H), 6.65 (m, 2H, Ar-H, *J* = 7.71 Hz), 2.27 (s, 6H, -CH$_3$, Ar-CH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 169.00, 164.33, 143.45, 138.97, 132.31, 131.84, 129.69, 128.32, 127.59, 126.90, 126.51, 125.15, 123.36, 116.64, 114.78, 110.19, 97.24, 67.45, 21.36, 17.15; ESI-MS (*m/z*): 567 $(M+1)^+$.

(1-(4-(4-(Dimethylamino)benzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl)phenyl)ethylidene)-2-oxo-2*H*-chromene-3-carbohydrazide (11c): Brick red powder; yield: 76%; m.p.: 282-284 ºC; IR (KBr, ν_{max}, cm^{-1}): 3340 (N-H *str.*), 3020 (Ar-H *str.*), 2889(aliphatic-CH *str.*), 1769 (C=O), 1680 (pyrone C=O *str.*), 1634 (C=N *str.*), 1490 (C=C *str.*), 1190 (C-O *str.*), 1065 (C-N *str.*); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 12.16 (s, 1H, -CONH), 8.40 (s, 1H, coumarin lactone), 8.11 (m, 9H, Ar-H), 7.79 (m, 6H, Ar-H), 7.42 (m, 4*H*, Ar-H & Ar-CH=), 3.10 (s, 6H, -N(CH$_3$)$_2$), 1.92 (s, 3H, -CH$_3$; ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 169.49, 163.24, 153.67, 153.50, 150.69, 141.36, 127.91, 127.82, 125.88, 120.95, 116.77, 107.57, 86.14, 40.34, 16.50; ESI-MS (*m/z*): 596 $(M+1)^+$.

(1-(4-(4-(Naphthalen-2-ylmethylene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl)phenyl)ethylidene)-2-oxo-2*H*-chromene-3-carbohydrazide (11d): Red colour powder; Yield: 69%; m.p.: 284-286 ºC; IR (KBr, ν_{max}, cm^{-1}): 3341 (N-H *str.*), 3158 (Ar-H *str.*), 2940 (aliphatic-CH *str.*), 1750 (C=O), 1699 (pyrone C=O *str.*), 1628 (C=N *str.*), 1461 (C=C *str.*), 1212 (C-O *str.*), 1037 (C-N *str.*); ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 12.20 (s, 1H, -CONH), 8.50 (s, 1H, coumarin lactone), 7.86 (m, 4*H*, Ar-H), 7.70 (m, 6H, Ar-H), 7.40 (m, 6H, Ar-H),

7.21 (d, 2H, Ar-H, *J* = 7.25 Hz), 6.83 (d, 2H, Ar-H), 1.90 (s, 3H, -CH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$, δ, ppm): 169.18, 166.46, 153.83, 144.46, 139.05, 131.23, 129.40, 128.35, 128.29, 127.53, 125.82, 120.73, 116.92, 109.00, 109.17, 16.08: ESI-MS (*m/z*): 603 $(M+1)^+$.

(1-(4-(4-Nitrobenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl)phenyl)ethylidene)-2-oxo-2*H*-chromene-3-carbohydrazide (11e): Brown colour powder; yield: 77%; m.p.: 270-272 ºC; IR (KBr, ν_{max}, cm^{-1}): 3352 (N-H *str.*), 3045 (Ar-H *str.*), 2910 (aliphatic-CH *str.*), 1740 (C=O), 1720 (pyrone C=O *str.*), 1629 (C=N *str.*), 1510 (C=C *str.*), 1234 (C-O *str.*), 1033 (C-N *str.*); ^{1}H NMR (300 MHz, CDCl$_3$, δ, ppm): 12.17 (s, 1H, -CONH), 8.42 (s, 1H, coumarin lactone), 7.90 (d, 2H, Ar-H), 7.70 (d, 2H, Ar-H), 7.30 (m, 4*H*, Ar-H), 6.90 (m, 5H, Ar-H), 6.50 (m, 6H, Ar-H), 1.89 (s, 3H, -CH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$, δ, ppm): 169.67, 163.24, 159.69, 154.67, 141.36, 127.91, 127.87, 125.88, 120.95, 116.77, 107.57, 16.91: ESI-MS (*m/z*): 598 $(M+1)^+$.

(1-(4-(4-(Furan-2-ylmethylene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl)phenyl)ethylidene)-2-oxo-2*H*-chromene-3-carbohydrazide (11f): Brick red; yield: 70%; m.p. (°C): 258-260; IR (KBr, ν_{max}, cm^{-1}): 3350 (N-H *str.*), 3023 (Ar-H *str.*), 2950 (aliphatic-CH *str.*), 1760 (C=O), 1682 (pyrone C=O *str.*), 1605 (C=N *str.*), 1467 (C=C *str.*), 1223 (C-O *str.*), 1028 (C-N *str.*); ^{1}H NMR (300 MHz, CDCl$_3$, δ, ppm): 12.10 (s, 1H, -CONH), 8.50 (s, 1H, coumarin lactone), 7.92 (d, 2H, Ar-H, *J* = 7.18 Hz), 7.32 (m, 4*H*, Ar-H), 7.07 (s, 1H, Ar-CH=), 6.86 (m, 5H, Ar-H), 6.50 (m, 6H, Ar-H), 2.42 (s, 3H, -CH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$, δ, ppm): 169.21, 164.46, 153.67, 153.50, 150.69, 141.36, 127.91, 127.82, 125.88, 120.95, 116.77, 107.57, 86.14, 16.39; ESI-MS (*m/z*): 542 $(M)^+$.

2-Oxo-(1-(4-(-5-oxo-2-phenyl-4-(thiophen-2-ylmethylene)-4,5-dihydro-1*H*-imidazol-1-yl)phenyl)ethylidene)-2*H*-chromene-3-carbohydrazide (11g): Brick red powder; Yield: 72%; m.p.: 260-262 ºC; IR (KBr, ν_{max}, cm^{-1}): 3408 (N-H *str.*), 3011 (Ar-H *str.*), 2910 (aliphatic-CH *str.*), 1747 (C=O), 1677 (pyrone C=O *str.*), 1610 (C=N *str.*), 1463 (C=C *str.*), 1241 (C-O *str.*), 1017 (C-N *str.*); ^{1}H NMR (300 MHz, CDCl$_3$, δ, ppm): 12.06 (s, 1H, -CONH), 8.69 (s, 1H, coumarin lactone), 8.16 (m, 5H, Ar-H), 7.80 (m, 5H, Ar-H), 7.6 (d, 2H, Ar-H), 7.40 (m, 6H, Ar-H & Ar-CH=), 7.20 (d, 2H, Ar-H, *J* = 8.35 Hz), 2.00 (s, 3H, -CH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$, δ, ppm): 169.23, 164.46, 153.93, 142.00, 128.58, 127.38, 125.79, 120.52, 116.76, 109.52, 106.40, 15.23; ESI-MS (*m/z*): 558 $(M)^+$.

(1-(4-(4-Fluorobenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl)phenyl)ethylidene)-2-oxo-2*H*-chromene-3-carbohydrazide (11h): Brick red; yield: 75%; m.p.: 254-256 ºC; IR (KBr, ν_{max}, cm^{-1}): 3390 (N-H *str.*), 3050 (Ar-H *str.*), 2810 (aliphatic-CH *str.*), 1745 (C=O), 1700 (pyrone C=O *str.*), 1620 (C=N *str.*), 1515 (C=C *str.*), 1234 (C-O *str.*), 1058 (C-N *str.*); ^{1}H NMR (300 MHz, CDCl$_3$, δ, ppm): 12.10 (s, 1H, -CONH), 8.50 (s, 1H, coumarin lactone), 7.50 (m, 15H, Ar-H), 7.08 (s, 1H, Ar-CH=), 6.37 (d, 2H, Ar-H), 1.82 (s, 3H, -CH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$, δ, ppm): 169.49, 164.08, 157.00, 153.98, 145.80, 137.48, 133.56, 129.13, 128.64, 127,88, 125.38, 120.61, 116.97, 105.07, 95.24, 16.11: ESI-MS (*m/z*): 572 $(M+2)^+$.

Biological assays

Disc diffusion assay: By utilizing the disc-diffusion method [14] and for bacteria Mueller-Hinton medium and for fungi the same medium with 4% glucose, the synthesized compounds (**11a-h**) were examined for their antimicrobial (antifungal as well as antibacterial) activities. In disc-diffusion technique, sterile paper discs (Ø 5 mm) were impregnated with chemical at concentrations of 100 µg/mL in DMSO. As a control, discs containing DMSO were utilized. The following suitable medium were used to spread the microorganism cultures: Sabouraud's agar for the yeast-like fungi (*C. albicans*) as well as Mueller–Hinton agar for *B. subtilis*, *S. aureus*, *E. coli* and *P. vulgaris* in Petri dishes. The plates were incubated at 35 ºC for 24 h for the microbial cultures. The growth inhibition zones surrounding the discs were visible after incubation, showing that the compound under research inhibits the development of microorganisms. In this research, every test was carried out three times. Standard medications included benzyl penicillin as well as fluconazole.

Microdilution assays: The microdilution broth technique was used to get the MIC (minimum inhibitory concentration) values for all the synthesized compounds. With the use of spectrophotometry, the suspensions optical density has been adjusted to 0.5 McFarland standard turbidity before the bacteria and fungal inocula were generated from 24 h broth cultures. The greatest concentration (1 mg/mL) of the test compounds that disintegrated in DMSO was first used. Usually, a serial two-fold dilution was generated to create various compound dilutions. Every dilution had a standard bacteria suspension and then added to fungus in a ratio of 1:1. The growth of bacteria was incubated at 37 ºC for around 24 h. Fluconazole and benzyl penicillin were used as reference drugs for the antimicrobial examinations. Control experiments were carried out using DMSO.

DPPH free radical scavenging activity: In 95% methanol, DPPH solution (0.004% w/v) was developed. For synthesis of stock solutions (10 mg/100 mL or 100 µg/mL), all of the test chemicals (**11a-h**) were mixed with 95% methanol. This mixture were taken in five test tubes 4, 2, 6, 10 and 8 mL of the final volume containing 10 mL whose concentration was then 40, 20, 60, 100 and 80 µg/mL respectively. In each of these test tubes, freshly produced DPPH solution was added and after 10 min, at 517 nm absorbance spectrum was measured by utilizing a spectrophotometer. The test for every sample was carried out three times. A reference standard was ascorbic acid [15,16].

The equation mentioned below was utilized to measure the % of the DPPH free radical that was scavenged:

$$\text{DPPH radical scavenging } (\%) = \frac{A_{control} - A_{test\ sample}}{A_{control}} \times 100$$

Assay for nitric oxide (NO) scavenging activity: By dissolving drug in a suitable solvent (dioxane/methanol) at a concentration of 100 µM and tubes were incubated at 25 ºC for 120 min. Sodium nitroprusside (5 mM) in phosphate buffer pH 7.4 was then added. A control experiment was maintained using the test compound and solvent in an equivalent quantity.

A 0.5 mL of the incubation solution was regularly removed and mixed with 0.5 mL of the Griess reagent. At 546 nm, the chromophore absorbance was created by diazotizing nitrite with sulphanilamide and then following up with N-naphthyl ethylenediamine was calculated. Three duplicate reports of the experiment were made.

Cytotoxic assay: Cell maintenance and culture according to the protocols described in the literature, the cytotoxicity of all recently produced hybrid compounds was assessed *in vitro* against a panel of human tumour cell lines. The panel includes cervical cancer (He La), breast carcinoma (MCF-7), lung carcinoma (A-549) and prostate cancer (DU-145).

Stock solutions of the test compound were prepared in DMSO. Following a 24 h incubation period, 48 h of incubation with various reagent concentrations up to 50 µM were added. The final DMSO concentration in each well was 0.01%. A other well that had only 0.01% of DMSO was used as a DMSO control, but it was determined to be inactive when in an conditions that were applied. The growth of cell that can be evaluated by utilizing MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma) reduction assay, relies on the capacity of viable cells to convert a soluble tetrazolium salt is of yellow colour into a blue formazan crystal. In a nutshell, all wells received 10 µL of MTT dye produced in PBS (phosphate buffered saline) 48 h after treatments began. The plates were then kept at 37 ºC for further 4 h. Each concentration was tested three times using the carefully collected supernatant from each well, formazan crystals dissolved in 100 µL of DMSO, measurements of absorbance at 540 nm wavelength.

$$\text{Cell toxicity (\% of control)} = \frac{\text{O.Dt} - \text{O.Db}}{\text{O.Dc} - \text{O.Db}} \times 100$$

where $O.D_b$ - mean optical density of blank wells, $O.D_t$ - mean optical density of treated wells, $O.D_c$ - mean optical density of control wells. The IC_{50} values were measured for the compounds concentration that possess growth of cell by 50% [17,18].

Molecular modelling

Molecular docking studies were performed using PyRx 0.8 software. The receptor molecules human Hsp90 (PDB ID: 5XQE) and human topoisomerase II (PDB ID: 1ZXN) were retrieved from protein data bank. The receptors 3D structures were made by utilizing Auto Dock 4.2 (MGLtools 1.5.6) by water molecules removal, co-crystallized ligands, cofactors and adding hydrogen bonds and adding charges. The compounds chemical structures were drawn utilizing the Chemoffice tool (Chemdraw 16.0) proper 2D orientation is assigned, energy minimization was done utilizing ChemBio3D.

Docking studies: The ligands with receptors docking were performed using pyrx software. All the targets were loaded in to the set as macromolecules as well as software. Then, using the PDBQT format, all of the ligands were loaded and docked with the macromolecule. Grid box was generated for 5XQE and 1ZXN with proper dimensions. The position of grid box: Hsp90 (X = 0.786, Y = 36.666, Z = -25.533) and topoisomerase II (X = 48.589, Y = -1.373, Z = 78.493). A maximum of 9 conformers were developed during the process of docking.

Visualization using discovery studio-visualizer: By choosing the conformations with the lowest free binding energy, interactions between ligands and target receptors were examined. Discovery Studio Visualizer examined interactions in 2D and 3D. Molecular docking scores of human against compounds were also studied. The residual interactions, binding score, hydrogen bonds and van der Waal interactions, of all the 8 compounds along with standard compounds against human Hsp90 (PDB ID: 5XQE) and Human type IIA DNA topoisomerase (PDB ID: 1ZXN) are computed.

***In silico* molecular and pharmacokinetic properties predictions:** The pharmacokinetic properties of the titled compounds were assessed using online servers SwissADME. Lipophilicity was calculated utilizing partition coefficient among *n*-octanol as well as water (log P_o/w) according to iLOGP, SILICOS-IT, MLOGP, XLOGP and WLOGP extrapolative models. The consensus log P is the arithmetic mean of the values anticipated by the 5 models. Solubility of water was estimated as the logarithm of the molar solubility in water (log S) utilizing the model SILICOS-IT extrapolative model. Druglikeness was predicted as per Lipinski and Verber rules. Properties like blood-brain (BBB) permeability, gastrointestinal (GI) absorption permeation, cytochrome p450 (CYP), substrate as well as inhibitor of permeability glycoprotein (P-gp) and skin permeation were also predicted.

RESULTS AND DISCUSSION

A series of 8 novel (4-(4-(arylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl)phenyl)ethylidene)-2-oxo-2*H*-chromene-3-carbohydrazides (**11a-h**) were synthesized by treating 4-(arylidene)-2-phenyloxazol-5(4*H*)-ones (**3a-h**) with *p*-aminoacetophenone (**4**) in the presence of DMF/$POCl_3$, yielded 1-(4'-acetylphenyl)-4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones (**5a-h**) which are key intermediates. 2-Oxo-2*H*-chromene-3-carbohydrazide (**10**) was another key intermediate which was prepared by the reaction of ethyl-2-oxo-2*H*-chromene-3-carboxylate (**8**) with hydrazine hydrate (**9**) in methanol at room temperature, whereas ethyl-2-oxo-2*H*-chromene-3-carboxylate (**8**) was prepared by the reaction of salicylaldehyde (**6**) with diethyl malonate (**7**) in existence of catalytic amounts of piperidine. The carbohydrazide (**10**) was then condensed with 1-(4-acetylphenyl)-4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones (**5a-h**) in ethanol for about 8 h furnished compounds (**11a-h**) (**Scheme-I**). The structures of the intermediates (**5a-h**) and the titled compounds (**11a-h**) were characterized by FT-IR, mass, ^1H & ^{13}C NMR spectral studies.

The IR spectra of compounds (**5a-h**) displays a band of absorption in the region of 3164-3020 cm^{-1} because aromatic stretching of C-H, 2996-2764 cm^{-1} because aliphatic C-H stretching, 1643-1790 cm^{-1} because of functional group -C=O, 1690-1540 cm^{-1} because of the –CH=CH- stretching and 1032-1016 cm^{-1} because of C-N stretching, showing the evidence that the intermediate compounds (**5a-h**) were formed. ^1H NMR spectra of compounds **5a-h** exhibited singlet at δ 2.15-2.50 because of $–CH_3$ protons and multiplets at δ 6.83-8.05 because aromatic and arylidene protons (Ar-H & Ar-CH=). Compound

5b showed singlet at δ 2.65 ppm because of Ar-C$\underline{H}_3$. Compound **5c** exhibited singlet at δ 3.20 ppm because of –N(C$\underline{H}_3$)$_2$ protons. Additionally, other protons were seen at the expected values of ppm. ^{13}C NMR spectrum of these compounds **5a-h** chemical shift value of carbon atoms that has been appeared at δ 26.29-27.51 ppm because of aliphatic –C$\underline{H}_3$, δ 161.46-166.88 ppm because of cyclic imine -$\underline{C}$=N, δ 166.88-169.76 ppm is attributed due to the cyclic amide –$\underline{C}$ONH and δ 197.69-198.51 ppm is due to aliphatic -$\underline{C}$=O functionality shows clear confirmation for the final product formation **5a-h**. Compound **5b** values of chemical shifts of a appeared atoms carbon δ 26.51 ppm is due to Ar-$\underline{C}$H$_3$, while compound **5c s**howed chemical shift value at δ 41.36 ppm (aliphatic, –N($\underline{C}$H$_3$)$_2$). Additionally, the mass spectra of compounds **5a-h** agrees with the suggested structures' stated molecular weight. Similarly, the final products **11a-h** were also well characterized.

Biological activity: The Gram-positive bacteria *S. aureus* MTCC96, *B. subtilis* MTCC121, yeast *C. albicans* MTCC3017 strains as well as the Gram-negative bacteria *P. vulgaris* MTCC 1771, *E. coli* MTCC739, were examined for their *in vitro* antibacterial activity against the titled compounds **11a-h**. The zone of inhibition was identified utilizing the filter paper disc technique for MIC using the broth micro-dilution method.

Compound **11b** (-CH$_3$), **11h** (*p*-fluoro) exhibit the potent activity of antibacterial whereas **11c** (*p*-N(CH$_3$)$_2$), **11e** (*p*-NO$_2$) showed the highest antifungal activity. Compounds with electron releasing substituents, such as compound **11b** (*p*-CH$_3$), have higher activity. Halogen substitution as in compound **11h** (-fluoro) showed increase in the activity. Compound **11a** (-H) exhibited equal activity against the bacterial strains to that of benzyl penicillin. However, nitro and naphthyl groups *i.e.* **11e** and **11d** diminished the activity. Antifungal activity findings reveal that **11c** (*p*-N (CH$_3$)$_2$), **11e** (*p*-NO$_2$) showed highest in activity. The remaining named compounds all shown strong against *C. albicans* action. Compounds **11b**, **11c** and **11h** exhibited lower MIC values (62.5 µg/mL) against *B. subtilis.* Compound **11h** displayed decreased MIC values (62.5 µg/mL) against *S. aureus.* Compound **11b** exhibited lower MIC values (62.5 µg/mL) against *E. coli*, while **11c** and **11e** exhibited the lowest MIC (62.5 µg/mL) for *C. albicans* (Table-1).

All the synthesized compounds were examined for the antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) technique and nitric oxide (NO) scavenging activity, anticancer activity by MTT method. Compounds **11a** (-H), **11d** (–naphthyl), **11g** (-thiophene) and **11h** (-fluoro) showed excellent antioxidant activity. Compound **11d** (–naphthyl) and **11f** (–furfural), exhibits the excellent anticancer activity of all the remaining compounds have displays moderate activities as well as good activities.

Results of DPPH scavenging activity (Table-2) represented that the title compounds (**11a-h**) can demonstrate substantial antioxidant action. Among which compounds **11a** and **11g** (-H and -thiophene) showed excellent antioxidant activity, whereas compound **11e** (*p*-NO$_2$) showed moderate activity, whereas in compound **11d** (-naphthyl) group reduced antioxidant action. All compounds have IC$_{50}$ values displayed in between the 24.8-96.26 µg/mL with antioxidant activity.

TABLE-1
ANTIMICROBIAL ACTIVITY OF SYNTHESIZED COMPOUNDS (**11a-h**)

Compound	Zone of inhibition (mm), MIC (µg/mL)				
	B. subtilis MTCC 441	*S. aureus* MTCC 96	*E. coli* MTCC 443	*P. vulgaris* MTCC 1771	*C. albicans* MTCC 183
11a	14 (125)	11 (250)	08 (500)	07 (500)	14 (125)
11b	18 (62.5)	10 (250)	16 (62.5)	07 (500)	13 (125)
11c	18 (62.5)	15 (125)	10 (250)	11 (250)	16 (62.5)
11d	08 (500)	06 (500)	NA	07 (500)	15 (125)
11e	09 (500)	15 (125)	NA	11 (250)	16 (62.5)
11f	11 (250)	11 (250)	10 (250)	11 (250)	11 (125)
11g	15 (125)	14 (250)	12 (250)	11 (250)	14 (125)
11h	18 (62.5)	17 (62.5)	07 (500)	07 (500)	07 (500)
Benzyl pencillin	16 (2)	17 (2)	12 (2)	13 (2)	–
Fluconazole	–	–	–	–	21 (8)

TABLE-2
ANTIOXIDANT ACTIVITY OF THE SYNTHESIZED COMPOUNDS (**11a-h**)

Compound	DPPH scavenging activity (%)					IC$_{50}$ (µg/mL)
	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL	
11a	45.23 ± 0.7	51.11 ± 0.1	67.39 ± 0.1	69.46 ± 0.5	71.48 ± 0.2	34.10
11b	37.59 ± 0.6	46.10 ± 0.6	51.92 ± 0.1	67.31 ± 0.6	70.50 ± 0.5	52.80
11c	37.13 ± 0.4	39.24 ± 0.6	47.82 ± 0.1	54.79 ± 0.3	68.13 ± 0.3	61.49
11d	31.23 ± 0.2	37.54 ± 0.4	48.05 ± 0.2	52.12 ± 0.2	63.70 ± 0.2	69.15
11e	36.13 ± 0.2	46.10 ± 0.6	51.92 ± 0.1	69.46 ± 0.5	73.46 ± 0.6	48.95
11f	47.22 ± 0.6	54.48 ± 0.2	67.34 ± 0.4	73.68 ± 0.5	81.87 ± 0.2	68.70
11g	36.13 ± 0.2	46.10 ± 0.6	51.92 ± 0.1	69.46 ± 0.5	73.46 ± 0.6	24.80
11h	31.02 ± 0.2	36.79 ± 0.2	41.83 ± 0.4	46.47 ± 0.3	50.25 ± 0.4	96.26
Ascorbic acid	55.12 ± 0.2	65.08 ± 0.2	75.26 ± 0.2	85.82 ± 0.4	93.74 ± 0.2	8.96

Ascorbic acid (reference antioxidant compounds) was used as a standard. The scavenging capacities were represented as percentage inhibition and values were the means of three replicates (mean ± SD, n = 3).

TABLE-3
ANTIOXIDANT ACTIVITY OF THE SYNTHESIZED COMPOUNDS (**11a-h**)

Compound	Nitric oxide scavenging activity (%)					IC_{50} (µg/mL)
	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL	
11a	40.23 ± 0.7	52.33 ± 0.7	68.48 ± 0.1	79.59 ± 0.2	85.48 ± 0.4	26.2
11b	35.42 ± 0.4	40.51 ± 0.2	46.82 ± 0.2	51.61 ± 0.3	57.83 ± 0.3	70.0
11c	34.11 ± 0.3	39.43 ± 0.4	41.78 ± 0.3	46.81 ± 0.1	53.10 ± 0.3	90.2
11d	47.19 ± 0.6	54.84 ± 0.2	68.43 ± 0.4	74.68 ± 0.5	81.87 ± 0.2	27.7
11e	36.23 ± 0.1	41.65 ± 0.4	44.11 ± 0.2	48.02 ± 0.2	54.90 ± 0.4	73.5
11f	31.23 ± 0.2	37.54 ± 0.4	48.05 ± 0.2	52.12 ± 0.2	63.70 ± 0.2	71.4
11g	37.13 ± 0.4	39.24 ± 0.6	47.82 ± 0.1	54.79 ± 0.3	68.13 ± 0.3	62.3
11h	43.41 ± 0.6	53.34 ± 0.2	70.54 ± 0.1	76.26 ± 0.1	85.21 ± 0.5	27.3
Ascorbic acid	55.12 ± 0.2	65.08 ± 0.2	75.26 ± 0.2	85.82 ± 0.4	93.74 ± 0.2	10.4

Ascorbic acid (reference antioxidant compounds) was used as a standard. The scavenging capacities were represented as percentage inhibition and values were the means of three replicates (mean ± SD, n = 3).

The results of the nitric oxide scavenging activity, as shown in Table-3, indicate that the title compounds (**11a-h**) possess antioxidant activity, which is clearly observed. Among which compounds **11a** (-H), **11d** (-naphthyl) and **11h** (-fluoro) showed an excellent antioxidant activity, whereas compound **11g** (-thiophene) showed good activity. Most of the compounds exhibited mild to moderate activity of antioxidant. All the synthesized compounds IC_{50} values lie between 26.2-90.2 µg/mL with antioxidant activity. These have demonstrated antioxidant activities on par with that of ascorbic acid.

All the titled compounds **11a-h** were screened for anticancer activity. Based on Table-4, compounds **11d**, **11f**, **11h** (-naphthyl, -furfural and -fluoro) showed excellent anticancer activity. Compounds **11a** and **11c** (-H and *p*-$N(CH_3)_2$) showed high activity against all the cell lines. Compounds **11b**, **11e** and **11g** (-CH_3, *p*-NO_2 and -thiophene) showed good activity. It was found that each compound made a remarkable anticancer activity than the standard doxorubicin.

TABLE-4
ANTICANCER ACTIVITY OF
SYNTHESIZED COMPOUNDS (**11a-h**)

Compounds	Lung cancer A-549 (µM)	Cervical cancer HeLa (µM)	Prostate cancer Du-145 (µM)	Liver cancer Hep-G2 (µM)
11a	0.03	0.03	0.03	0.03
11b	0.04	0.04	0.04	0.04
11c	0.03	0.03	0.03	0.03
11d	0.01	0.01	0.01	0.01
11e	0.05	0.05	0.05	0.05
11f	0.01	0.01	0.01	0.01
11g	0.04	0.04	0.04	0.04
11h	0.01	0.01	0.04	0.01
Doxorubicin	0.06	0.06	0.07	0.07

Molecular modeling studies: Specific structure modeling and accurate activity prediction are the two goals of molecular docking investigations [19]. The interaction in between compounds and active sites of the human Hsp90 and human topoisomerase II target proteins was investigated using the PyRx 0.8 software. The synthesized compounds' molecular docking investigation was done to determine their patterns of binding with Human Hsp90 (PDB ID: 5XQE) and it was compared to standard antifungal fluconazole and standard anticancer agent doxorubicin. The minimum binding energies ranges from (-11 to -12.7 kcal/mol), among all the compounds **11d** (-12.7 kcal/mol) and **11f** (-12.4 kcal/mol) showed good binding affinity with the minimum binding energies (Table-5). As shown in Figs. 1 and 2, compounds **11d** and **11f** were well inserted into the active pocket of Hsp90. The binding interactions of fluconazole with human Hsp90 (PDB ID: 5XQE), binding interactions of doxorubicin with human Hsp90 (PDB ID: 5XQE) respectively is shown in Figs. 3 and 4. Compound **11f** by forming bonds of hydrogen with amino acid sequence of TYR139, THR184 to improve the affinity with Hsp90. Human topoisomerase II (PDB ID: 1ZXN) was selected as another target protein. The molecular docking analysis of the compounds exhibit minimum binding energies (Figs. 5 and 6) from (-10.4 to -11.5 kcal/mol), among which compounds **11d** (-11.5 kcal/mol) and **11e** (-11.4 kcal/mol) showed good binding affinity comparatively with doxorubicin (Fig. 7). Compounds **11d** and **11e** established a hydrogen bond with an amino acid sequence ASN150, LYS168 respectively to enhance the affinity with Human Type IIA DNA topoisomerase (Table-6). The synthesized derivatives may successfully be inserted into the active pocket of Hsp90, according to a molecular docking analysis, Human Type IIA DNA topoisomerases.

***In silico* physico-chemical and pharmacokinetic properties prediction:** The pharmacokinetic and physico-chemical properties prediction was predicted by employing online free web server SwissADME tools. Table-7 displays the SwissADME predicted lipophilicity, solubility of water, drug likeness and bio-availability values of the synthesized compounds. The values of Log P as high as 6.08 (**11d**) and 5.65 (**11b**), the least value being 4.43 (**11e**). Compounds **11d**, **11e** and **11h** were low soluble while the remaining compounds are highly soluble. Compounds **11g** and **11h** violate only 1 rule given by Lipinski, whereas the remaining compounds violate two rules. Moreover, all the compounds pass the Veber's rule except compound **11e**. The bioavailability score of the compounds **11a-f** is 0.17, while compounds **11g** and **11h** it is 0.55.

Table-8 depicts the outcome of prediction by pharmacokinetics of the titled compounds. Skin permeation scores (log Kp in cm/s) of the synthesized compounds ranged from -4.4

TABLE-5
MOLECULAR DOCKING SCORES OF HUMAN Hsp90 (PDB ID: 5XQE) AGAINST SYNTHESIZED COMPOUNDS (**11a-h**)

Compounds	Binding affinity (kcal/mol)	Hydrogen bonds	Pi-pi T-shaped/pi-pi stacked/pi-alkyl/pi-sigma/alkyl/amide pi-stacked/pi-sulfur/carbon hydrogen bond/pi-anion/pi-cation/pi-donor hydrogen bond interactions	van der Waal interactions
11a	-11.8	Asn-51, Gly-135	Lys-58, Ala-55, Phe-138, Trp-162, Val-150	Tyr-61, Asp-57, Ile-96, Met-98, Asp-54, Ile-107, Ile-110, val-136, Ala-111, Tyr-139
11b	-11.2	–	Asn-51, Ala-55, Met-98, Trp-162, Val-150, Leu-103, Phe-138, Leu-107, Asp-57, Lys-58	Gly-135, Tyr-61, Thr-184, Asp-54
11c	-11.9	Tyr-139, Leu-107	Tyr-61, Asp-57, Lys-58, Asp-54, Ala-55, Met-98, Phe-138, Val-150	Ile-96, Asn-51, Gly-108, Gly-135, Ile-110, Ala-111, Val-136, Trp-162, Ile-103
11d	-12.7	–	Tyr-139, Trp-162, Leu-107, Ala-55, Asp-57, Lys-58	Phe-22, Val-150, Phe-138, Met-98, Asn-51, Ile-96, Asp-54, Gly-108, Ala-111, Gly-135, Ile-110, Tyr-61
11e	-11	Leu-107, Gly-108, Tyr-139	Ile-110, Asp-54, Lys-58, Val-150, Met-98	Val-135, Gly-135, Phe-138, Ala-111, Asn-51, Ala-55, tyr-162, Ile-103, Asp-102
11f	-12.4	Tyr-139, Thr-184	Phe-22, Ala-111, Leu-107, Phe-138, Met-98, Ala-55, Lys-58	Gly-135, Val-136, Asp-54, Gly-97, Ile-96, Val-186, Val-150, Trp-162, Ile-26, Gln-23, Gly-108, Phe-170, Ile-104, Ile-103, Asn-51
11g	-11.5	Lys-58	Leu-107, Tyr-139, Phe-138, Trp-162, Val-150	His-54, -104, Ile-96, Asp-54, Ala-55, Gly-108, Val-136, Ala-111, -104, Ile-103, Gly-135, Met-98
11h	-12.1	Tyr-139, Gly-108	Lys-158, Ile-96, Leu-107, Phe-138, Trp- 162, Met-98, Val-150	Asn-51, Asp-102, Tyr-61, Ala-55, Asp-57, Asp-54, Asn-51, Asp-102, Gly-97, Yal-136, -104, Ile-103, Ala-111, Gly-135
Fluconazole	-8.1	Tyr-139, Trp-162	Phe-138, Val-150, Met-98, Val-136, Ala-111, Leu-107	Gly-135, Gly-108, Phe-170, Leu-103, Phe-22, Thr-184, Val-186, Asn-51
Doxorubicin	-9.8	Asp-93, Ser-52, Asn-51	Ile-110, Leu-107, Phe-138, Asp-54	Ala-55, Thr-184, Met-98, Gly-135, Ala-111, Tyr-139, Gly-108, Val-150, Asp-102, Trp-162, Leu-103, Leu-48, Ile-91, Leu-48, Val-186

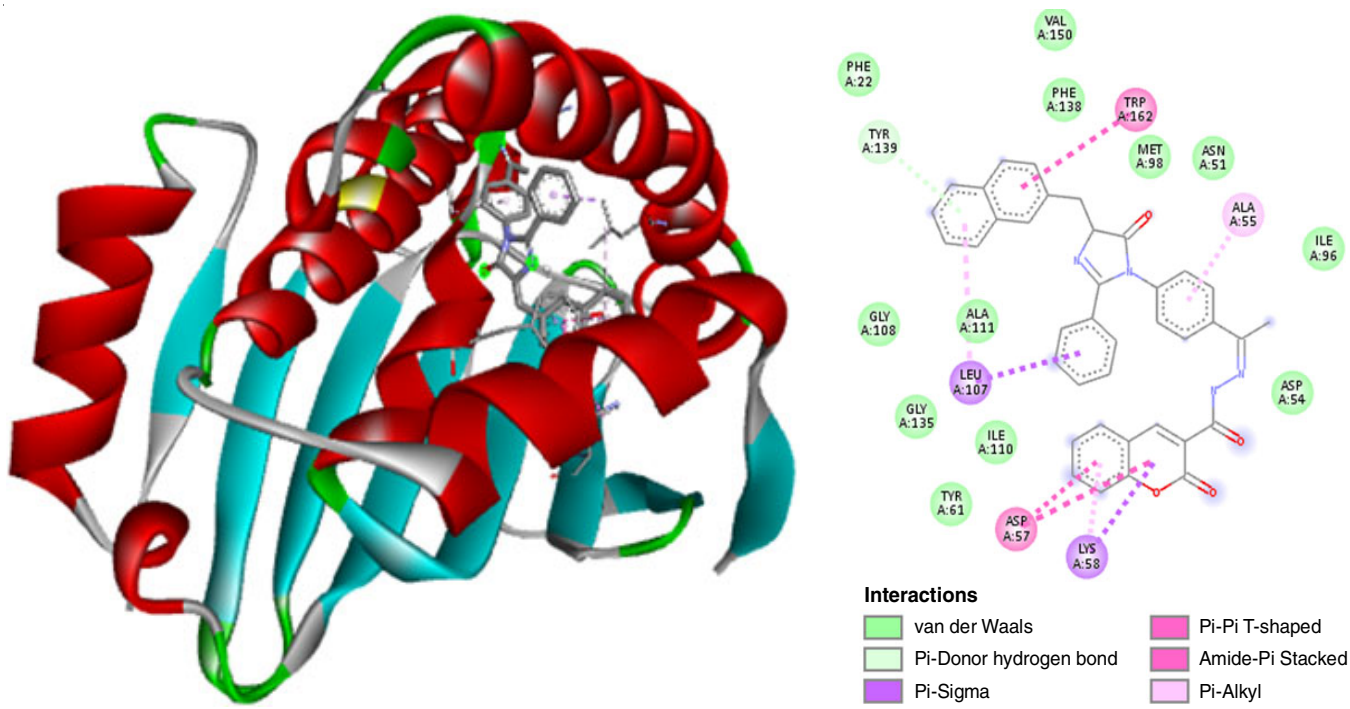


Fig. 1. Binding interactions of compound **11d** with human Hsp90 (PDB ID: 5XQE)

to 5.56. The synthesized compounds that are apart from **11d**, **11e** and **11h** have GI absorption that is high and none of the compounds displayed the blood-brain barrier permeability. All the compounds are not substrates of Pgp. None of the compound is inhibitor of CYP1A2 and CYP2D6. Compound **11h** is predicted to be inhibitors of CYP3A4, **11a**, **11g** and **11h** are inhibitors of CYP2C19. Compounds **11a**, **11c**, **11g** and **11h** are inhibitors of CYP2C9.

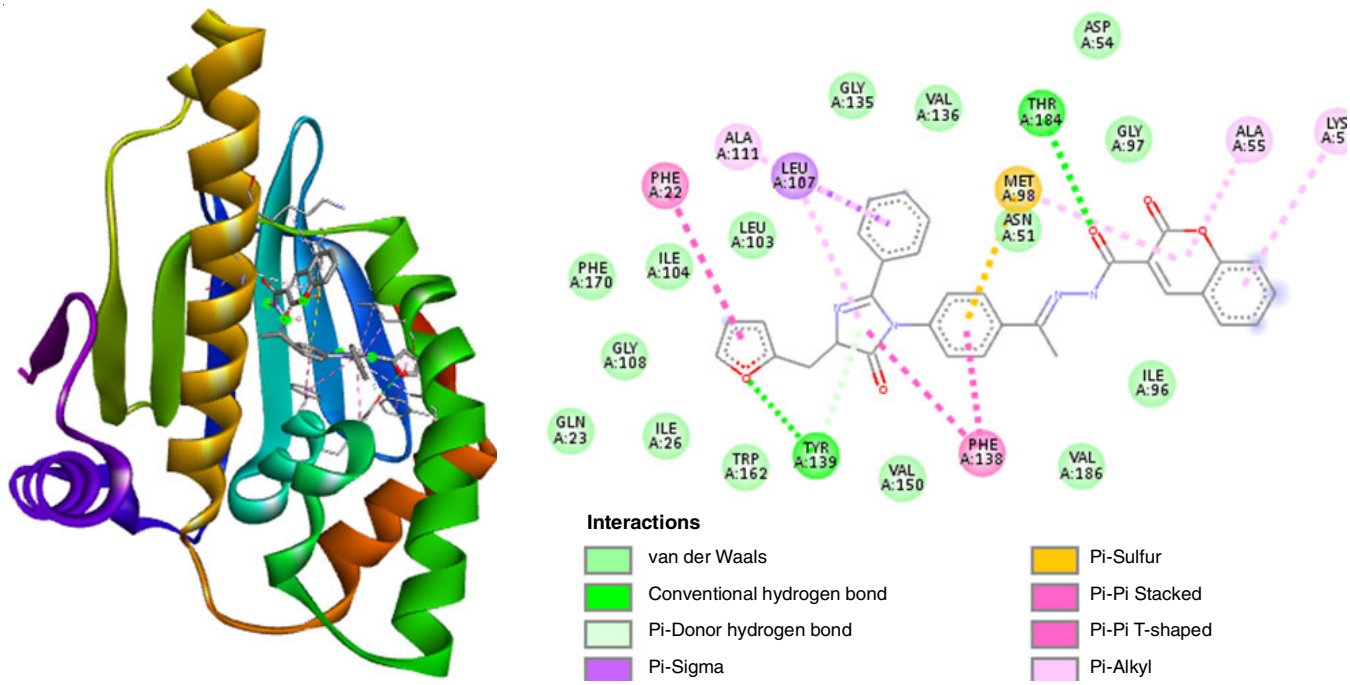


Fig. 2. Binding interactions of compound **11f** with human Hsp90 (PDB ID: 5XQE)

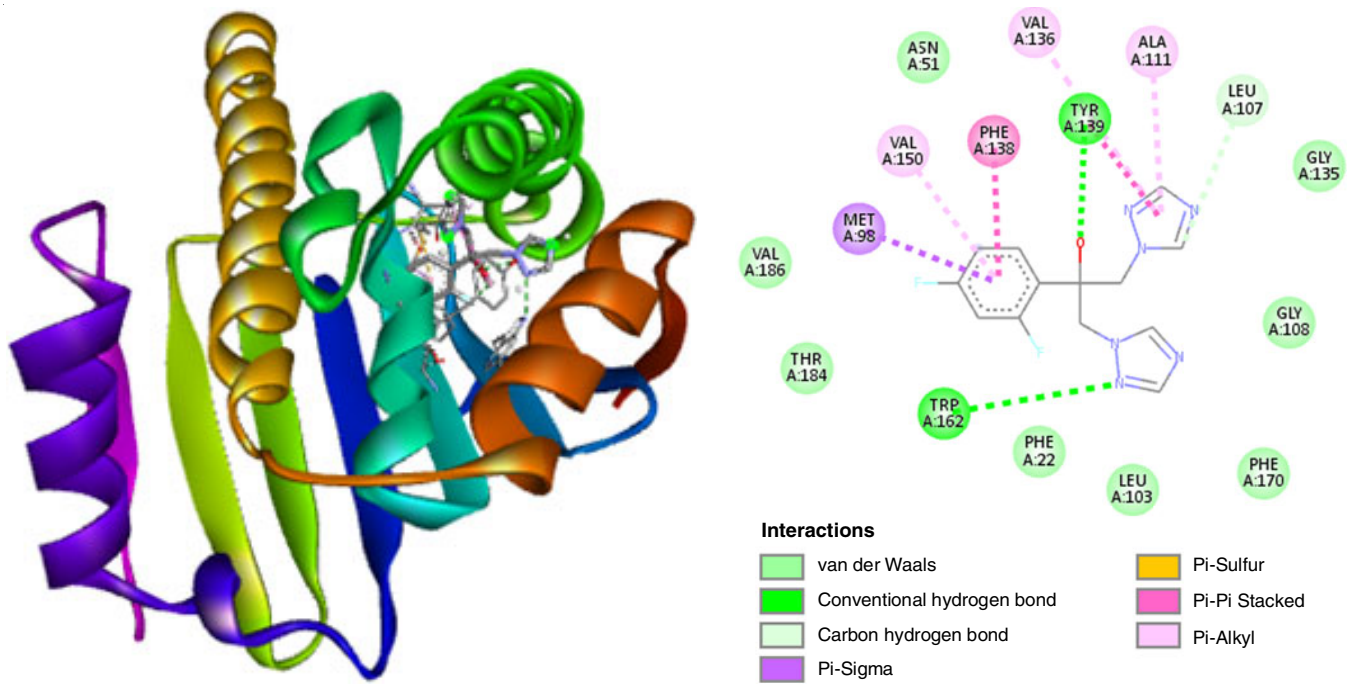


Fig. 3. Binding interactions of compound fluconzole with human Hsp90 (PDB ID: 5XQE)

Drug-likeness estimate, which is based on a physico-chemical or structural analysis of compounds in late-stage drug development, is a qualitative evaluation of oral bioavailability. As per the Lipinski's rule, an orally active substance must have the following properties: not more than (NMT) 5 hydrogen bond donors (HBD), NMT 10 HBD, a molecular weight below 500 g/mol and a log P LT < 5. Even if a molecule breaks two or more of the rules, it would not be inactive when taken orally. Only compounds **11g** and **11h** fulfil the criteria for oral bio-availability in accordance with these criteria.

However, all the synthesized compounds obey the Veber's rule involving of only 2 criteria: 10 or less rotatable bonds as well as polar surface (TPSA) area not more than 140 Å^2. The compounds' 0.17 bio-availability value indicates that they have a 17% chance of at least 10% oral bioavailability in rats or human colon carcinoma (Caco-2) permeability that are detectable; as a result, they won't be readily bioavailable when taken orally. However, the compounds' 0.55% bioavailability number corresponds to a chance of 55%, thus it's feasible that these compounds will work better as oral medications. It has been

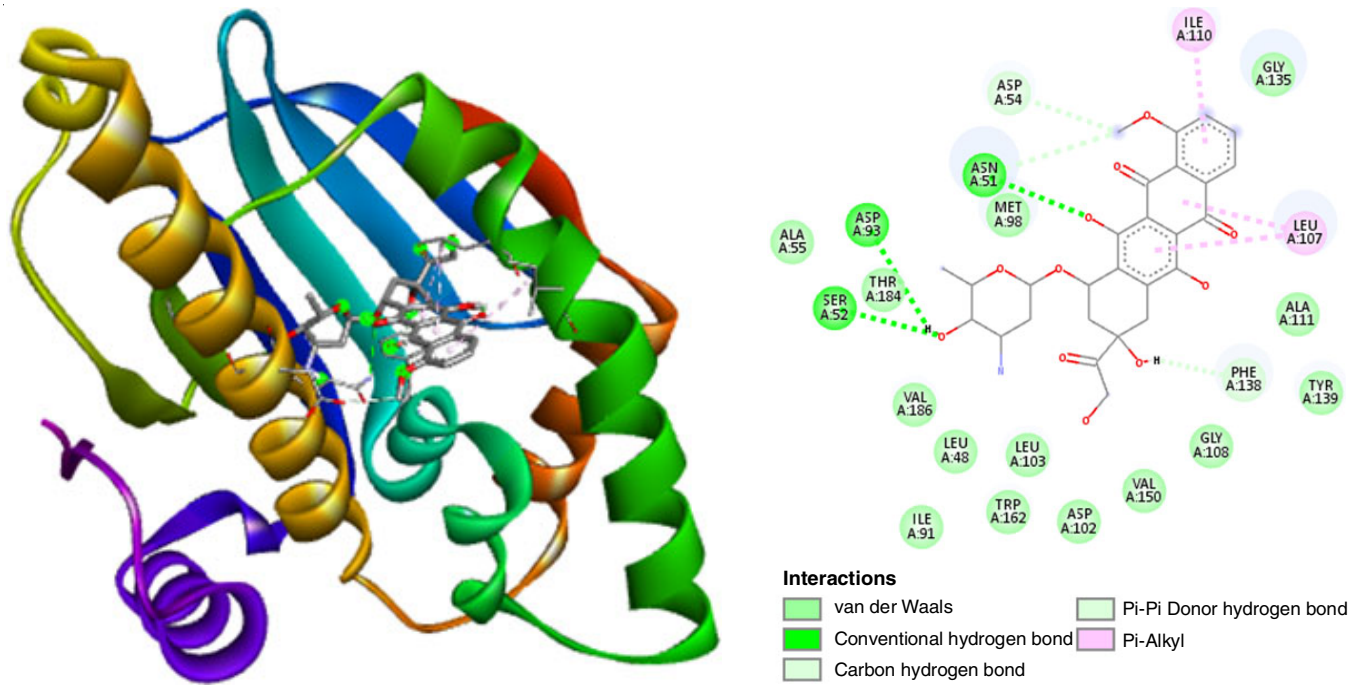


Fig. 4. Binding interactions of compound doxorubicin with human Hsp90 (PDB ID: 5XQE)

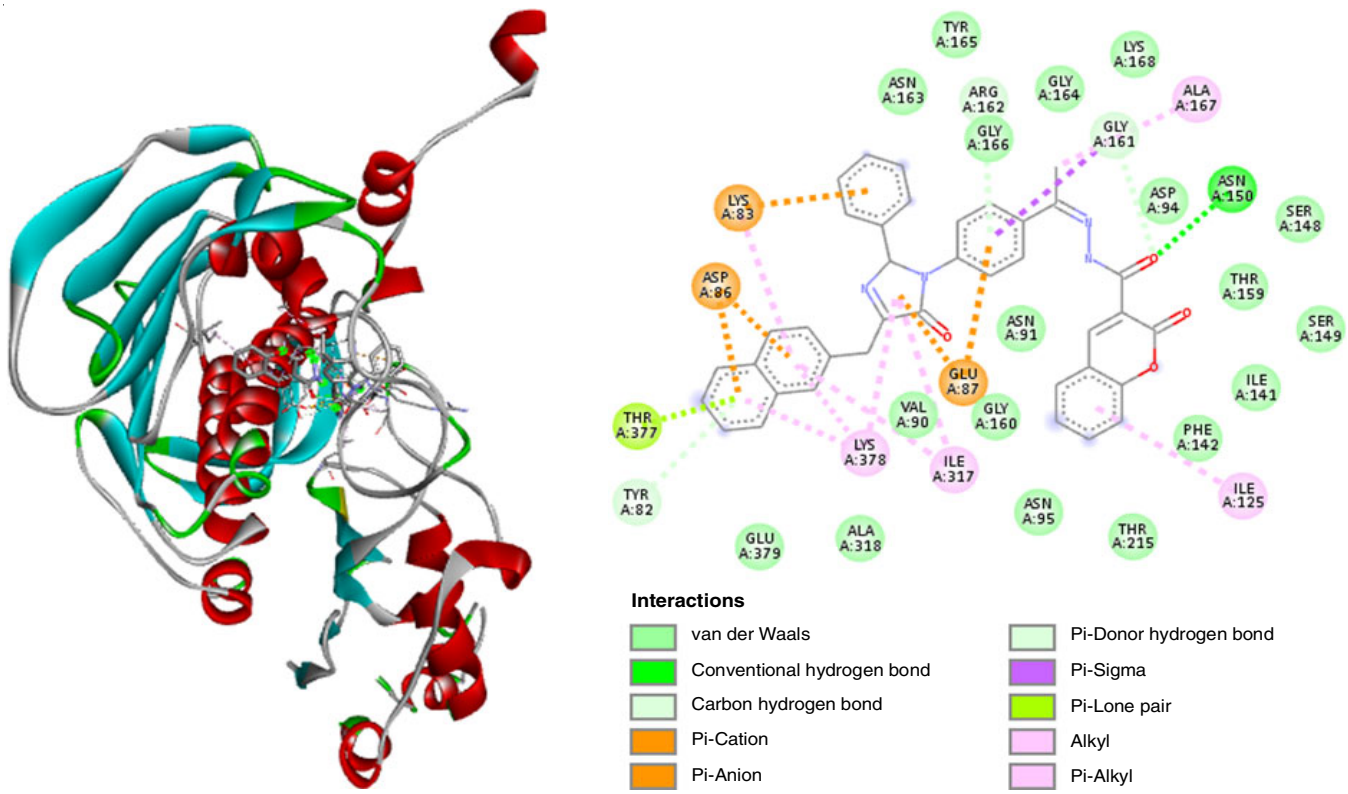


Fig. 5. Binding interactions of compound **11d** with human type IIA DNA topoisomerases (PDB ID: 1ZXN)

estimated that inhibition of CYP enzymes is a main reason of drug-drug interactions. Similarly, Pgp substrates, are likely to be denied access to their chosen action site [20].

Conclusion

The SAR investigations of the synthesized coumarin imidazole hybrids for antibacterial as well as antifungal analysis discloses that the coumarin and imidazole molecules are very promising target compounds. The substituents on the aryl group, which is in conjugation with imidazolone moiety were more effective on antimicrobial activity. Molecular docking studies confirmed that the synthesized derivatives could be effectively inserted into the active pocket of Human Hsp90 and Human type IIA DNA topoisomerase. The current findings proposed

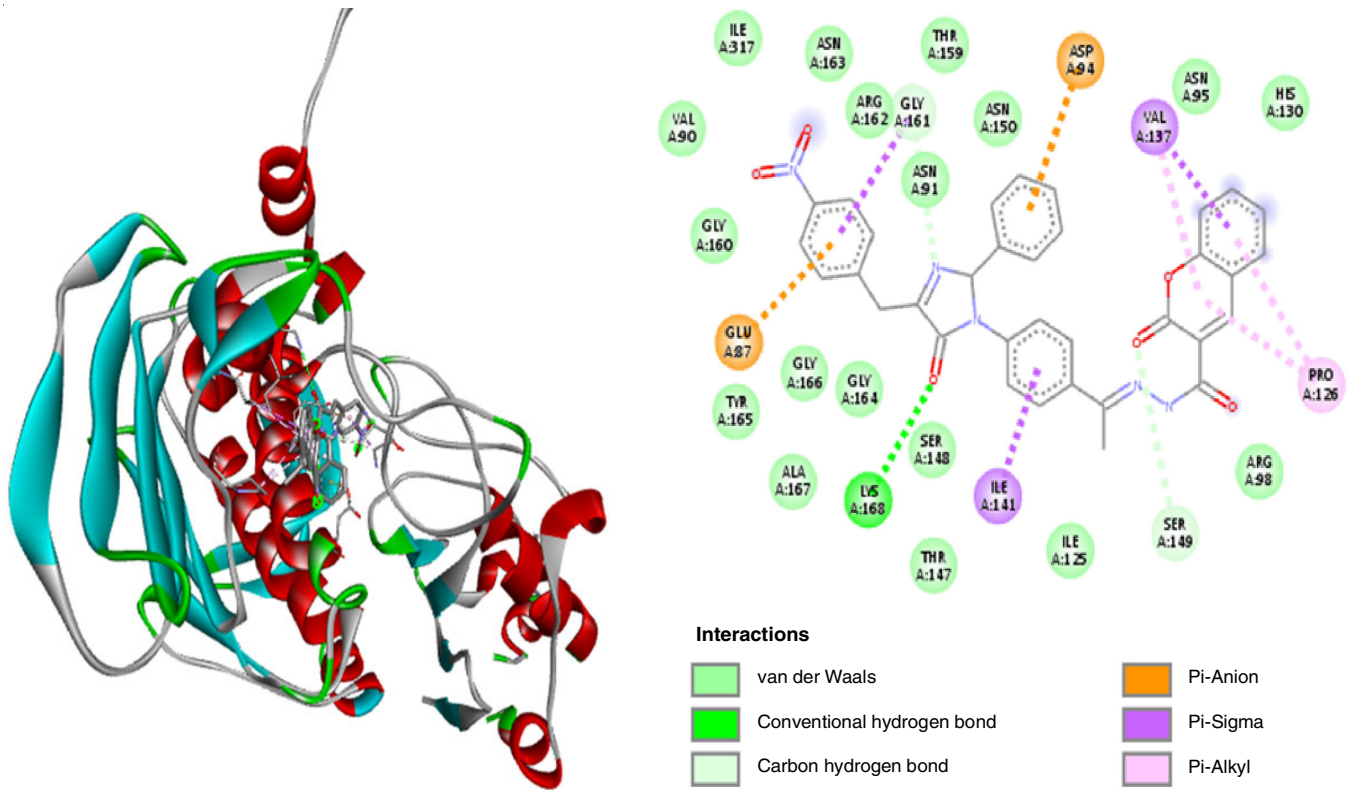


Fig. 6. Binding interactions of compound **11e** with human type IIA DNA topoisomerases (PDB ID: 1ZXN)

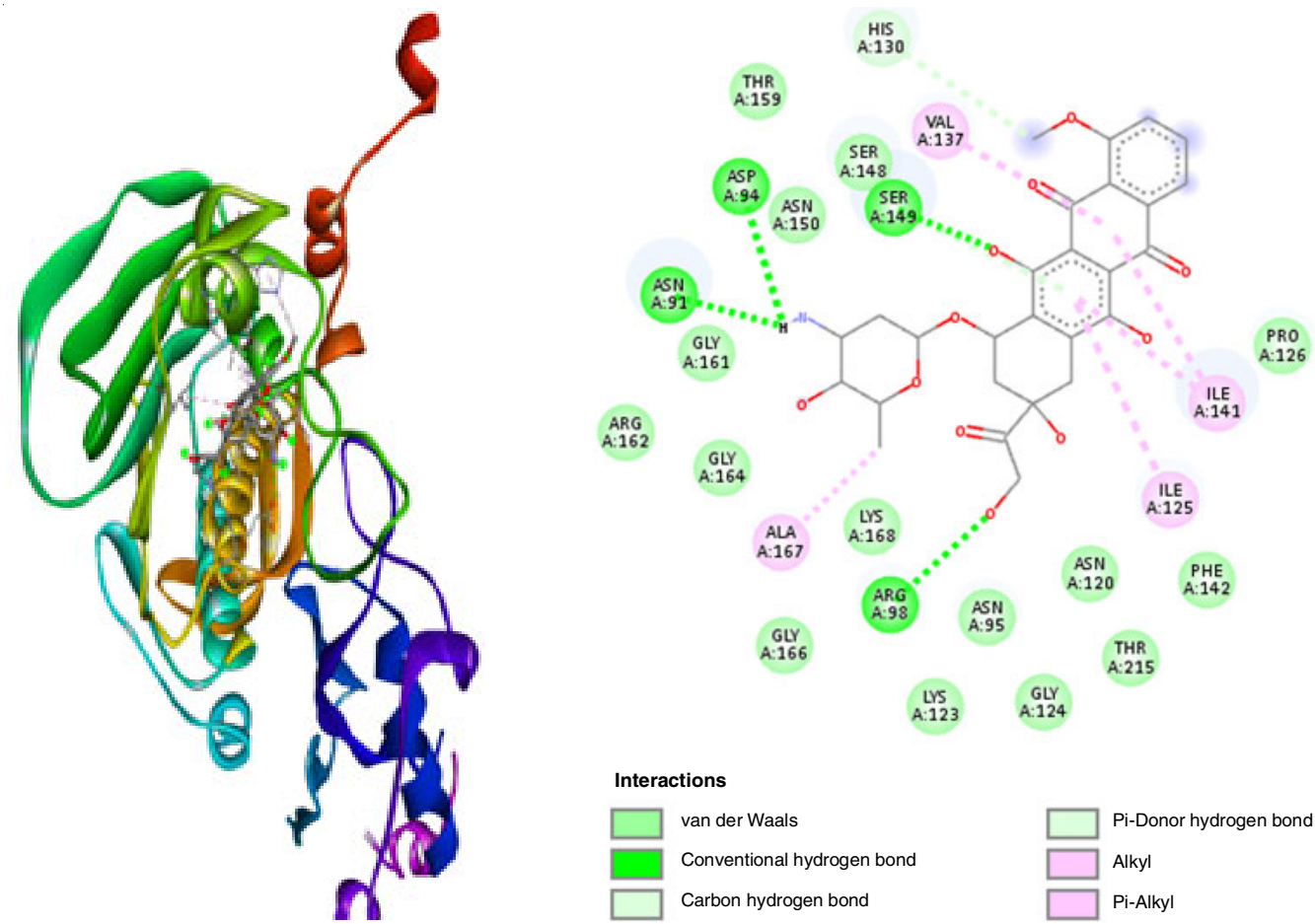


Fig. 7. Binding interactions of compound doxorubicin with human type IIA DNA topoisomerases (PDB ID: 1ZXN)

TABLE-6
MOLECULAR DOCKING SCORES OF HUMAN TYPE IIA DNA TOPOISOMERASE (PDB ID: 1ZXN) AGAINST SYNTHESIZED COMPOUNDS (**11a-h**)

Compounds	Binding affinity (kcal/mol)	Hydrogen bonds	Pi-pi T-shaped/pi-pi stacked/pi-alkyl/pi-sigma/alkyl/amide pi-stacked/pi-sulfur/carbon hydrogen bond/pi-anion/pi-cation/pi-donor hydrogen bond/pi-lone pair*/halogen (fluorine) **interactions	van der Waal interactions
11a	-11.3	Asn-150, Asn-95	Val-137, Pro-126, Ser-149, Arg-98, Ile-141, Ile-125, Asn-91, Ala-167, Phe-142	His-130, Asp-94, Gly-161, Lys-168, Ser-148, Gly-133, Leu-140, Thr-147
11b	-10.5	Asn-150	Tyr-186, Pr0-126, Arg-98, Val-128, Val-137, Ile-141, His-130, Ala-167, Gly-164	Ile-125, Ser-148, Gly-161, Lys-168, Asn-91, Gly-166, Arg-162, Lys-131, Glu-129
11c	-10.7	Ser-149	Asn-91, Glu-87, Ala-167, Ile-125, Arg-98, Val-137, Pro-126	His-130, Val-128, Tyr-186, Asn-120, Thr-215, Asn-95, Ala-92, Phe-142, Ser-148, Asn-150, Gly-166, Ile-141, Lys-168, Gly-161, Asn-163, Gly-164, Arg-162, Tyr-165
11d	-11.5	Asn-150	Ala-167, Gly-161, Arg-162, Ile-125, Glu-87, Lys-378, Ile-317, Lys-83, Asp-86, Tyr-82, Thr-377*	Gly-379, Ala-318, Val-90, Gly-160, Asn-91, Asn-95, Thr-215, Phe-142, Ile-141, Ser-149, Thr-159, Ser-148, Asp-94, Lys-168, Gly-164, Gly-166, Asn-163, Tyr-165
11e	-11.4	Lys-168	Gly-161, Asp-94, Val-137, Ser-149, Pro-126, Ile-141, Glu-87	Asn-95, His-130, Arg-98, Ile-125, Thr-147, Ser-148, Gly-164, Gly-166, Tyr-165, Ala-167, Gly-160, Val-90, Ile-317, Asn-163, Arg-162, Thr-159, Asn-91, Asn-150
11f	-10.7	Ser-149	Ile-141, Val-137, Leu-140	Thr-159, Pro-126, Asp-94, ASn-150, Phe-142, Asn-91, Thr-215, Ala-92, Asn-120, Asn-95, Ile-125, Arg-98, Ser-148, Thr-147, His-130, Glu-133
11g	-10.4	Arg-98	His-130, Ile-141, Phe-142, Ile-217, Ala-92, Ile-125, Ile-88	Asn-91, Lys-168, Leu-140, Thr-147, Ser-148, Val-137, Ser-149, Pro-126, Asn-95, Thr-215, Asn-120
11h	-10.8	Arg-98	Ile-88**, Ile-141, Phe-142, His-130, Ser-149, Ile-125, Ile-217, Thr-215, Ala-92	Asn-95, pro-126, Leu-140, Ser-148, Val-137, Thr-147, Lys-168, Ala-167, Asn-120, Asn-91, Ile-118
Doxorubicin	-8.9	Asn-94, Asn-91, Ser-149, Arg-98	His-130, Val-137, Ile-141, Ile-125, Ala-167	Pro-126, Phe-142, Asn-120, Thr-215, Gly-124, Asn-120, Asn-95, Gly-124, Lys-123, Lys-168, Gly-166, Gly-164, Arg-162, Gly-161, Asn-150, Ser-148, Thr-159

TABLE-7
PREDICTED LIPOPHILICITY (log P), WATER SOLUBILITY (log Sw), DRUGLIKENESS AND BIOAVAILABILITY SCORES OF SYNTHESIZED COMPOUNDS (**11a-h**)

Compd.	m.w.	NHBD	NHBA	NRTB	TPSA	Consensus log P	Log Sw (Silicos-IT)	Solubility class	Lipinski #violations	Veber #violations	Bioavail-ability score
11a	552.58	1	6	7	104.34	5.07	-11.52	High	2	0	0.17
11b	566.61	1	6	7	104.34	5.65	-11.89	High	2	0	0.17
11c	595.65	1	6	8	107.58	5.37	-11.58	High	2	0	0.17
11d	602.64	1	6	7	104.34	6.08	-13.13	Low	2	0	0.17
11e	597.58	1	8	8	150.16	4.43	-10.84	Low	2	1	0.17
11f	570.57	1	7	7	104.34	5.62	-11.78	High	2	0	0.17
11g	542.54	1	7	7	117.48	4.73	-10.75	High	1	0	0.55
11h	558.61	1	6	7	132.58	5.35	-10.78	Low	1	0	0.55

TABLE-8
PHARMACOKINETICS PREDICTION OF SYNTHESIZED COMPOUNDS (**11a-h**)

Compd.	GI absorption	Blood-brain permeability	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Skin permeation log Kp (cm/s)
11a	High	No	No	No	Yes	Yes	No	No	-4.98
11b	High	No	No	No	No	No	No	No	-4.81
11c	High	No	No	No	No	Yes	No	No	-5.16
11d	Low	No	No	No	No	No	No	No	-4.4
11e	Low	No	No	No	No	No	No	No	-5.37
11f	High	No	No	No	No	No	No	No	-5.02
11g	High	No	No	No	Yes	Yes	No	No	-5.56
11h	Low	No	No	No	Yes	Yes	No	Yes	-5.22

that coumarin derivatives conjugated with imidazole could be promising inhibitors for target proteins. We have successfully designed and synthesized efficiently novel coumarin linked imidazolone derivatives. Among them **11b**, **11h** exhibited potent antibacterial and **11c**, **11e** exhibited greater antifungal activity. Compounds **11a**, **11d**, **11g** and **11h** also showed excellent antioxidant activity. Moreover, **11d** and **11f** showed equipotent anticancer activity. All the compounds should significant molecular properties and pharmacokinetic properties *in silico*. This class of compounds certainly can serve as novel and potential templates in medicinal chemistry.

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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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