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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antitubercular activity, and QSAR analysis of substituted nitroaryl analogs: chalcone, pyrazole, isoxazole, and pyrimidines

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Abstract In the present investigation, 4-nitroacetophenone, on condensation with appropriate aldehydes in ethanolic sodium hydroxide solution, yielded the corresponding chalcones. These corresponding chalcones were reacted with hydrazine hydrate, urea, thiourea, and hydroxylamine hydrochloride, which led to the formation of corresponding novel pyrazole, pyrimidine, and isooxazole derivatives, respectively. All newly synthesized compounds were evaluated for their antimycobacterial activities against *Mycobacterium tuberculosis* using agar dilution. The results indicated that most of the compounds demonstrated better in vitro antitubercular activity, and the compound **1g** has a MIC of 1.56 μg mL^{-1}. QSAR analysis revealed that molecular descriptors mean electrotopological state (Ms) and average valance connectivity index chi-0 ($^{0}\chi_{Av}$) contributed positively while 3-path Kier alpha-modified shape index ($^{3}\kappa_{\alpha}$) contributed negatively. The objective of our study is to generate new leads and to optimize their structure to display the potent efficacy.

Keywords Tuberculosis · Chalcones · Antimycobacterial · QSAR · SQ-MLR

Introduction

The design as well as identification of new molecules for the treatment of diseases such as the tuberculosis and cancer is an important undertaking in medicinal chemistry research. The chemistry of chalcones has generated intensive scientific interest due to their biological and industrial applications. Chalcones are natural biocides and are well-known intermediates in the synthesis of heterocyclic compounds exhibiting various biological activities. The extensive literature survey was carried out to find out the different types of molecular structures which have been developed till date and methodologies used for rational designing of anti-mycobacterial agents. The literature review reveals that chalcone derivatives exhibit diverse pharmacological activities such as antimicrobial (Prasad *et al.*, 2008), anticancer (Jevwon *et al.*, 2005), antitubercular (Shivakumar *et al.*, 2005), and antiviral (Churkin *et al.*, 1982) etc.

The α, β-unsaturated ketonic group which is responsible for bactericidal activity of chalcones is also of great use in further chemical modification into various heterocyclic moieties. Therefore, chalcones are considered as most important molecules for the synthesis of various heterocyclic systems. The diverse properties of chalcones have prompted us to synthesize them to study their antimycobacterial activity.

Tuberculosis (TB) is by far the most frequently encountered mycobacterial disease in the world. Mycobacteria are ubiquitous organisms that are becoming increasingly important intracellular pathogens that establish an infection in oxygen-rich macrophages of the lung (O'Brien and Nunn, 2001). About 32 % of the world's population is currently infected with TB. Every year, approximately eight to nine million of these infected people develop clinical pulmonary TB leading to nearly three million annual deaths (Corbett *et al.*, 2003; Dye *et al.*, 1999; World Health Organization, 2003). If the present trend continues, TB is likely to claim more than 30 million lives within the next decade (WHO Report, 2007).

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The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious disease and the increasing number of multidrug-resistant microbial pathogens. In spite of a large number of antibiotics and chemotherapeutics available for medical use, at the same time, the emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new classes of antimicrobial agents (Kaplancikli *et al.*, 2008). Moreover, the emergence of multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis*, which is insensitive to one or more of the first-line drugs, isoniazid and rifampicin, has further worsened the situation (Alland *et al.*, 1994; Jacobs, 1994; Weltman and Rose, 1994). Furthermore, the association of TB and HIV infections has caused an urgent need in search of alternative chemotherapeutics for *M. tuberculosis* infection (Lin *et al.*, 2001, 2002).

It is well known that no new anti-TB drug has been discovered in the last 40 years. A major concern is the rise of multidrug-resistant-TB (MDR-TB); fatality rates are much higher with MDR-TB and they are up to 100-fold more expensive to treat. Even more serious is the emergence of extensively drug-resistant TB (XDR-TB) that has been confirmed in more than 45 countries. This increase of MDR-TB and the emergence of XDR-TB provide the rationale to search for new antimycobacterial drugs (Gupta and Kaskhedikar, 2011). Therefore, in order to control the rapid spread of TB, there is an urgent need for new anti-TB drugs with unique modes of action and improved properties such as enhanced activity against MDR strains, reduced toxicity and shortened duration of therapy (Global Alliance for TB Drug Development, 2001; Smith *et al.*, 2004, Duncan, 2003).

Our research efforts toward the development of novel anti-TB agents are in the direction of discovering new classes of compounds, which are structurally different from known anti-TB drugs. Several structural classes of antituberculosis agents includes substituted chalcones, coumarines, pyrimidines, quinolones etc. which are reported to exhibit promising activities against drug-resistant and drug-sensitive strains of tuberculi (Nayyar and Jain, 2005). To the best of our knowledge, there has been no previous report of various nitro heterocyclic derivatives and parent chalcones as antitubercular agents. However, there are numerous examples of nitrogen-containing heterocycles being used to treat TB, for example clofazimine, isoniazid, and pyrazinamide.

The antitubercular activity of nitroaryl/heteroaryls is mainly due to the metabolic reduction of their nitro group by a class of enzymes called nitroreductases. The nitroreductase family comprises a group of flavin mononucleotide or flavin adenine dinucleotide-dependent enzymes that are able to metabolize nitroaromatic and nitroheterocyclic derivatives (nitrosubstituted compounds) using the reducing power of nicotinamide adenine dinucleotide (NAD(P)H). Microorganisms with appropriate nitroreductases act on nitroaryls to produce a highly reactive electrophilic intermediate and this is postulated to affect DNA as the reduced intermediates of nitroaryls do. Other evidence suggests that the reduced nitroaryls bind to bacterial ribosomes and prevent protein synthesis (Edwards, 1993; Bryant *et al.,* 1981).

Several in silico techniques are utilized in the process of drug design and development of antitubercular agents. One such technique is quantitative structure activity relationship (QSAR), has been traditionally perceived as means of establishing correlation between trends in chemical structure modification and respective changes of biological activity (Yao *et al.*, 2004). This quantitative technology can be utilized to improve the structure of the inhibitor molecule and to interpret the improved structure in terms of favorable biological interactions (Hansch and Leo, 1995). Thus, the use of predictive computational (in silico) QSAR models allows the biological properties of virtual structures to be predicted, and a more informed choice of target to be selected for synthesis (Painea *et al.*, 2010). The use of computational approaches for the estimation of the activity of various molecules as drug candidates prior to their synthesis can save the resources and accelerate the drug discovery procedure (Zhoua *et al.*, 2007). We carried out QSAR analysis and established QSAR models to guide further structural optimization and predict the potency and physiochemical properties of clinical drug candidates.

The current work describes the synthesis of novel chalcone moiety and their structural modification into various heterocyclic derivatives like pyrazole, isoxazole, oxopyrimidine, and thiopyrimidine, etc., with respect to their antimycobacterial activity against *M. tuberculosis* and their QSAR analysis has been explored.

Experimental

All the chemicals used in the synthesis of compounds were of synthetic grade, and they were procured from Sigma, SD-fine, Himedia and E. Merck. All the melting points were taken in open capillaries tube and are uncorrected. The purity of compounds was checked routinely by thin-layer chromatography (TLC) (0.2-mm thickness) using silica gel, G-coated aluminum, plates (Merck), and spots were visualized by exposing the dry plates in iodine vapors and UV detector (long and short wavelength). As quantitative yields of chalcone derivatives 1–5 were obtained, neither crystallization nor flash column chromatographic purification (using silica gel # 240-400 mesh) is necessary. Infrared (IR) spectra (λ_{max} in cm^{-1}) were recorded on an Perkin Elmer FT-IR using KBr technique. ^{1}H-nuclear

magnetic resonance (NMR) spectra were observed on a BRUKER DRX-300 (300 MHz) NMR instrument using dimethyl sulfoxide (DMSO)-d_6 as solvent and TMS as internal reference (chemical shifts in δ ppm). Mass spectra were obtained on a JEOL SX 102/DA-6000 instrument using fast atom bombardment (FAB) technique.

General procedure

General synthesis of chalcones (**1a**–**1i**, **6** and **7**)

Substituted aromatic aldehydes (10 mmol) and *p*-nitro acetophenone (10 mmol) were dissolved in ethanol (25 mL) and the mixture was cooled to 5–10 °C in ice bath. 50 % sodium hydroxide solution (2.5 mL) was added slowly to the reaction mixture with constant stirring. Reaction mixture was stirred for another 2–4 h until the entire mixture becomes very cloudy. The progress of the reaction was monitored through TLC. The mixture was kept overnight in refrigerator. Reaction mixture was poured slowly into crushed ice, acidified with dilute hydrochloric acid. The precipitate was filtered, washed with ice-cold water, dried, and purified by flash column chromatography.

*3-(3-Methoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**1a**)*

M.p. 116–118 °C; Yield—80 %; IR (ν in cm^{-1}): 1657 (C=O), 1602 (Ar–C=C), 1516 (asym, Ar–NO_2), 1340 (sym, Ar–NO_2), 1256 (–OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.37–8.31 (d, 2H, Ar–H, $J = 16.8$ Hz), 8.16–8.13 (d, 2H, Ar–H, $J = 9$ Hz), 7.84–7.78 (d, 1H, CH=CH, $J = 17.1$ Hz), 7.53–7.48 (d, 1H, CH=CH, $J = 15.3$ Hz), 7.43–6.99 (m, 4H, Ar–H), 3.87 (s, 3H, OCH_3); FAB–MS: *m/z* 283 (M^+).

*3-(4-Methoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**1b**)*

M.p. 172–174 °C; Yield—81 %; IR (ν in cm^{-1}): 1655 (C=O), 1595 (Ar–C=C), 1526 (asym, Ar–NO_2), 1333 (sym, Ar–NO_2), 1247 (–OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.38–8.29 (m, 4H, Ar–H), 7.89–7.83 (d, 2H, CH=CH, $J = 16.5$ Hz), 7.79–7.75 (d, 2H, Ar–H, $J = 12$ Hz), 7.04–7.02 (d, 2H, Ar–H, $J = 6$ Hz), 3.83 (s, 3H, OCH_3); FAB–MS: *m/z* 283 (M^+).

*3-(4-Chlorophenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**1c**)*

M.p. 154–156 °C; Yield—72 %; IR (ν in cm^{-1}): 3097 (CH), 1662 (C=O), 1606 (Ar–C=C), 1520 (asym, Ar–NO_2), 1332 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.38 (s, 4H, Ar–H), 8.02–7.95 (m, 3H, Ar–H and CH=CH), 7.83–7.77 (d, 1H, CH=CH, $J = 15.5$ Hz), 7.57–7.54 (m, 2H, Ar–H); FAB–MS: *m/z* 288 ($M+H^+$).

*3-(3-Chlorophenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**1d**)*

M.p. 106–108 °C; Yield—70 %; IR (cm^{-1}) (ν in cm^{-1}): 3102 (CH), 1667 (C=O), 1603 (Ar–C=C), 1520 (asym, Ar–NO_2), 1341 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.37-8.34 (d, 4H, Ar–H, $J = 9$ Hz), 7.95-7.87 (m, 3H, Ar–H and CH=CH), 7.63-7.47 (m, 3H, Ar–H and CH=CH); FAB–MS: *m/z* 288 ($M+H^+$).

*3-(3,4-Dimethoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**1e**)*

M.p. 176–178 °C; Yield—65 %; IR (ν in cm^{-1}): 2969 (CH), 1660 (C=O), 1604 (Ar–C=C), 1515 (asym, Ar–NO_2), 1346 (sym, Ar–NO_2), 1236 (OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.39–8.32 (m, 4H, Ar–H), 7.84–7.73 (m, 2H, Ar–H), 7.56–7.55 (d, 1H, CH=CH, $J = 3.3$ Hz), 7.44–7.42 (d, 1H, CH=CH, $J = 6$ Hz), 7.05–7.03 (d, 1H, Ar–H, $J = 6.1$ Hz), 3.86 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3); FAB–MS: *m/z* 314 ($M+H^+$).

*3-(2,5-Dimethoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**1f**)*

M.p. 118–120 °C; Yield—68 %; IR (ν in cm^{-1}): 2971 (CH), 1661 (C=O), 1602 (Ar–C=C), 1512 (asym, Ar–NO_2), 1341 (sym, Ar–NO_2), 1238 (OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.39–8.34 (m, 4H, Ar–H), 7.56–7.54 (d, 1H, CH=CH, $J = 6$ Hz), 7.45–7.42 (d, 1H, CH=CH, $J = 9$ Hz), 6.95–6.79 (m, 3H, Ar–H), 3.77 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3); FAB–MS: *m/z* 314 ($M+H^+$).

*3-(2,4-Dichlorophenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**1g**)*

M.p. 200–202 °C; Yield—75 %; IR (ν in cm^{-1}): 3096 (CH), 1666 (C=O), 1596 (Ar–C=C), 1520 (asym, Ar–NO_2), 1331 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.39 (s, 4H, Ar–H), 8.29–8.27 (d, 1H, Ar–H, $J = 6$ Hz), 8.08–8.02 (m, 2H, Ar–H) 7.78–7.77 (d, 1H, CH=CH, $J = 3$ Hz), 7.60-7.57 (d, 1H, CH=CH, $J = 9$ Hz); FAB–MS: *m/z* 322 ($M+H^+$).

*3-(4-Fluorophenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**1h**)*

M.p. 194–196 °C; Yield—75 %; IR (ν in cm^{-1}): 1609 (Ar–C=C), 1510 (asym, Ar–NO_2), 1334 (sym, Ar–NO_2),

1160 (Ar–F); ^{1}H NMR DMSO (δ in PPM): 8.40–8.33 (m, 4H, Ar–H), 8.03–8.00 (m, 2H, Ar–H), 7.90 (s, 1H, CH=CH), 7.83 (s, 1H, CH=CH), 7.36–7.30 (m, 2H, Ar–H); FAB–MS: *m/z* 272 ($M+H^+$).

*3-(3,4,5-Trimethoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**1i**)*

M.p. 158–160 °C; Yield—71 %; IR (ν in cm^{-1}): 2968 (CH), 1668 (C=O), 1602 (Ar–C=C), 1507 (asym, Ar–NO_2), 1323 (sym, Ar–NO_2), 1250 (OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.40–8.33 (m, 4H, Ar–H), 7.90–7.86 (d, 1H, CH=CH, $J = 12$ Hz), 7.76–7.72 (d, 1H, CH=CH, $J = 12.2$ Hz), 7.26 (s, 2H, Ar–H), 3.86 (s, 6H, OCH_3), 3.72 (s, 3H, OCH_3); FAB–MS: *m/z* 344 ($M+H^+$).

General synthesis of pyrazoles (**2a**–**2j** and **8**)

Hydrazine hydrate (10 mmol), substituted chalcones (**1a**–**1i**, 10 mmol) and sodium acetate (10 mmol) were taken in ethanol (12 mL) and mixture was refluxed for 6–8 h. The progress of the reaction was monitored through TLC. After completion of the reaction, mixture was concentrated under reduced pressure and poured into ice water. The precipitate was filtered, washed with water, and dried. The crude product was purified by silica gel column chromatography to afford the corresponding pyrazole derivatives (**2**).

*5-(3-Methoxyphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**2a**)*

M.p. 224–226 °C; Yield—65 %; IR (ν in cm^{-1}): 3308 (NH), 2955 (C–H), 1596 (Ar–C=C), 1506 (asym, Ar–NO_2), 1336 (sym, Ar–NO_2), 1257 (–OCH_3);^{1}H NMR DMSO (δ in PPM): 8.40 (bs, 1H, NH), 8.32–8.29 (d, 2H, Ar–H, $J = 9$ Hz), 8.15–8.11 (m, 2H, Ar–H), 8.04–8.01 (d, 1H, Ar–H, $J = 9.1$ Hz), 7.52–7.36 (m, 2H, Ar–H), 6.95–6.93 (d, 1H, Ar–H, $J = 6$ Hz), 5.01-4.96 (dd, 1H, CH, $J = 3.6$, 11.1 Hz), 3.67 (s, 3H, OCH_3), 3.52–3.48 (dd, 1H, CH_2, $J = 3.6$, 10.8 Hz), 2.94–2.86 (dd, 1H, CH_2, $J = 4.4$, 17.3 Hz); FAB–MS: *m/z* 298 ($M+H^+$).

*5-(4-Methoxyphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**2b**)*

M.p. 124–126 °C; Yield—67 %; IR (ν in cm^{-1}): 3318 (NH), 2953 (C–H), 1600 (Ar–C=C), 1509 (asym, Ar–NO_2), 1339 (sym, Ar–NO_2), 1253 (–OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.34–8.29 (m, 3H, Ar–H and NH), 8.14–8.09 (m, 2H, Ar–H), 7.78–7.73 (m, 2H, Ar–H), 6.95–6.93 (d, 2H, Ar–H, $J = 6$ Hz), 4.99–4.95 (dd, 1H, CH, $J = 4.2$, 10.2 Hz), 3.73 (s, 3H, OCH_3), 3.51–3.48 (dd, 1H, CH_2, $J = 4.1$, 7.2 Hz), 2.96–2.88 (dd, 1H, CH_2, $J = 4.8$, 17.2 Hz); FAB–MS: *m/z* 298 ($M+H^+$).

*5-(4-Chlorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**2c**)*

M.p. 162–164 °C; Yield—62 %; IR (ν in cm^{-1}): 3310 (NH), 3056 (CH), 1599 (Ar–C=C), 1504 (asym, Ar–NO_2), 1337 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.25 (bs, 1H, NH), 8.23–8.19 (d, 2H, Ar–H, $J = 11.4$ Hz), 7.84–7.81 (d, 2H, Ar–H, $J = 8.7$ Hz), 7.74–7.69 (m, 2H, Ar–H), 7.30–7.26 (m, 2H, Ar–H), 5.01–4.97 (dd, 1H, CH, $J = 4.4$, 9.9 Hz), 3.54–3.49 (dd, 1H, CH_2, $J = 3.9$, 13.2 Hz), 2.95–2.88 (dd, 1H, CH_2, $J = 4$, 17.4 Hz); FAB–MS: *m/z* 302 ($M+H^+$).

*5-(3-Chlorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**2d**)*

M.p. 186–188 °C; Yield—61 %; IR (ν in cm^{-1}): 3314 (NH), 3081 (CH), 1594 (Ar–C=C), 1507 (asym, Ar–NO_2), 1339 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.38–8.10 (m, 3H, Ar–H and NH), 7.94–7.80 (m, 2H, Ar–H), 7.58–7.20 (m, 4H, Ar–H), 5.03–4.95 (dd, 1H, CH, $J = 4$, 18.1 Hz), 3.54–3.48 (dd, 1H, CH_2, $J = 4.2$, 12.9 Hz), 3.10–3.02 (dd, 1H, CH_2, $J = 4.4$, 17.2 Hz); FAB–MS: *m/z* 302 ($M+H^+$).

*5-(3,4-Dimethoxyphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**2e**)*

M.p. 89–91 °C; Yield—66 %; IR (ν in cm^{-1}): 3339 (NH), 2953 (CH), 1593 (Ar–C=C), 1507 (asym, Ar–NO_2), 1307 (sym, Ar–NO_2), 1257 (–OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.38–8.20 (m, 3H, Ar–H and NH), 7.83–7.80 (d, 2H, Ar–H, $J = 9$ Hz), 6.99–6.85 (m, 3H, Ar–H), 4.96–4.88 (dd, 1H, CH, $J = 4.3$, 17.8 Hz), 3.85–3.80 (dd, 1H, CH_2, $J = 5.7$, 8.4 Hz), 3.78 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 2.97–2.89 (dd, 1H, CH_2, $J = 4.2$, 17.5 Hz); FAB–MS: *m/z* 328 ($M+H^+$).

*5-(2,5-Dimethoxyphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**2f**)*

M.p. 150–152 °C; Yield—64 %; IR (ν in cm^{-1}): 3364 (NH), 1595 (Ar–C=C), 1533 (asym, Ar–NO_2), 1339 (sym, Ar–NO_2), 1259 (–OCH_3);^{1}H NMR DMSO (δ in PPM): 8.19–8.17 (d, 2H, Ar–H, $J = 6$ Hz), 8.09 (bs, 1H, NH), 7.81–7.79 (d, 2H, Ar–H, $J = 5.7$ Hz), 6.95–6.79 (m, 3H, Ar–H), 5.14–5.08 (dd, 1H, CH, $J = 7.3$, 10.4 Hz), 3.76 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.51–3.44 (dd, 1H, CH_2, $J = 4.8$, 15.6 Hz), 2.81–2.74 (dd, 1H, CH_2, $J = 8.1$, 15.3 Hz); FAB–MS: *m/z* 328 ($M+H^+$).

*5-(2,4-Dichlorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**2g**)*

M.p. 88–90 °C; Yield—68 %; IR (ν in cm^{-1}): 3281 (NH), 2951 (CH), 1595 (Ar–C=C), 1506 (asym, Ar–NO_2), 1336 (sym, Ar–NO_2), 1080 (Ar–Cl); ^{1}H NMR $CDCl_3$ (δ in PPM): 8.28–8.24 (d, 2H, Ar–H, $J = 11.4$ Hz), 7.79–7.76 (d, 2H, Ar–H, $J = 9$ Hz), 7.57–7.54 (d, 1H, Ar–H, $J = 9.3$ Hz), 7.43–7.42 (d, 1H, Ar–H, $J = 3.6$ Hz), 7.26 (s, 1H, Ar–H), 5.40–5.33 (dd, 1H, CH, $J = 8.5$, 12.3 Hz), 3.72–3.66 (dd, 1H, CH_2, $J = 4.4$, 13.1 Hz), 2.98–2.81 (dd, 1H, CH_2, $J = 9.8$, 11.8 Hz); FAB–MS: *m/z* 336 ($M+H^+$).

*5-(4-Fluorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**2h**)*

M.p. 140–142 °C; Yield—64 %; IR (ν in cm^{-1}): 3277 (NH), 1600 (Ar–C=C), 1506 (asym, Ar–NO_2), 1333 (sym, Ar–NO_2), 1230 (Ar–F); ^{1}H NMR DMSO (δ in PPM): 8.25 (bs, 1H, NH), 8.22–8.19 (d, 2H, Ar–H, $J = 8.7$ Hz), 7.83–7.80 (d, 2H, Ar–H, $J = 9$ Hz), 7.74–7.39 (m, 2H, Ar–H), 7.20–7.15 (m, 2H, Ar–H), 5.00–4.95 (dd, 1H, CH, $J = 4.6$, 10.7 Hz), 3.54–3.47 (dd, 1H, CH_2, $J = 4.1$, 16.1 Hz), 2.94–2.87 (dd, 1H, CH_2, $J = 8.6$, 11.6 Hz); FAB–MS: *m/z* 286 ($M+H^+$).

*3-(4-Nitrophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole (**2i**)*

M.p. 148–150 °C; Yield—68 %; IR (ν in cm^{-1}): 3354 (NH), 2943 (CH), 1592 (Ar–C=C), 1506 (asym, Ar–NO_2), 1328 (sym, Ar–NO_2), 1235 (–OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.24–8.21 (m, 3H, Ar–H and NH), 7.84–7.81 (d, 2H, Ar–H, $J = 8.7$ Hz), 6.71 (s, 2H, Ar–H), 4.97–4.82 (dd, 1H, CH, $J = 9.6$, 12.6 Hz), 3.77 (s, 6H, OCH_3), 3.64 (s, 3H, OCH_3), 3.54–3.49 (dd, 1H, CH_2, $J = 4.8$, 12 Hz), 3.00–2.90 (dd, 1H, CH_2, $J = 11.4$, 16.5 Hz); FAB–MS: *m/z* 358 ($M+H^+$).

*5-(4-Bromophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole (**2j**)*

M.p. 138–140 °C; Yield—60 %; IR (ν in cm^{-1}): 3334 (NH), 1595 (Ar–C=C), 1506 (asym, Ar–NO_2), 1338 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.33–8.24 (m, 3H, Ar–H and NH), 7.83–7.80 (d, 2H, Ar–H, $J = 9$ Hz), 7.57–7.55 (d, 2H, Ar–H, $J = 6.3$ Hz), 7.35–7.32 (d, 2H, Ar–H, $J = 9.6$ Hz), 5.00–4.94 (dd, 1H, CH, $J = 7.9$, 12 Hz), 3.57–3.52 (dd, 1H, CH_2, $J = 4.7$, 12.2 Hz), 2.95–2.86 (dd, 1H, CH_2, $J = 11.4$, 15.5 Hz); FAB–MS: *m/z* 346 ($M+H^+$).

General synthesis of dihydropyrimidinone derivatives (**3a**–**3j** and **9**)

Substituted chalcone (**1a**–**1i**) (20 mmol) and urea (20 mmol) were dissolved in ethanol (20 mL) and ethanolic sodium hydroxide (0.2 g in 2.5 mL) was added to reaction mixture. The mixture was refluxed for 8–10 h with constant stirring. The progress of the reaction was monitored through TLC. After completion of reaction, mixture was cooled and poured into ice-cold water with constant stirring for an hour. The mixture was kept in refrigerator for 24 h. The precipitate was filtered, washed with water, dried. The crude product was purified by silica gel column chromatography to afford the corresponding pyrimidin-2(1*H*)-one derivatives (**3**).

*6-(3-Methoxyphenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidin-2(1H)-one (**3a**)*

M.p. 185–187 °C; Yield—67 %; IR (ν in cm^{-1}): 3174 (NH), 2968 (C–H), 1647 (C=O), 1595 (Ar–C=C), 1520 (asym, Ar–NO_2), 1311 (sym, Ar–NO_2), 1258 (–OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.63–8.35 (m, 3H, Ar–H and NH), 8.20–8.16 (d, 2H, Ar–H, $J = 12.3$ Hz), 7.56–7.39 (m, 3H, Ar–H), 7.26–7.20 (m, 1H, Ar–H), 5.11–5.03 (m, 2H, CH_2), 4.76 (m, 1H, CH), 3.74 (s, 3H, OCH_3); FAB–MS: *m/z* 326 ($M+H^+$).

*6-(4-Methoxyphenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidin-2(1H)-one (**3b**)*

M.p. 204–206 °C; Yield—70 %; IR (ν in cm^{-1}): 3184 (NH), 2973 (C–H), 1653 (C=O), 1595 (Ar–C=C), 1507 (asym, Ar–NO_2), 1339 (sym, Ar–NO_2), 1255 (–OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.6 (s, 1H, NH), 8.32–8.26 (d, 2H, Ar–H, $J = 17.4$ Hz), 8.17–8.14 (m, 2H, Ar–H), 7.78–7.72 (m, 2H, Ar–H), 6.95–6.91 (d, 2H, Ar–H, $J = 11.6$ Hz), 5.23–5.11 (m, 2H, CH_2), 4.76 (m, 1H, CH), 3.73 (s, 3H, OCH_3); FAB–MS: *m/z* 326 ($M+H^+$).

*6-(4-Chlorophenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidin-2(1H)-one (**3c**)*

M.p. 166–168 °C; Yield—71 %; IR (ν in cm^{-1}): 3195 (NH), 2935 (C–H), 1652 (C=O), 1593 (Ar–C=C), 1521 (asym, Ar–NO_2), 1339 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.41 (bs, 1H, NH), 8.32–8.21 (m, 4H, Ar–H), 8.02–7.95 (m, 2H, Ar–H), 7.57–7.54 (m, 2H, Ar–H), 5.12–5.07 (m, 2H, CH_2), 4.85 (m, 1H, CH); FAB–MS: *m/z* 330 ($M+H^+$).

*6-(3-Chlorophenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidin-2(1H)-one (**3d**)*

M.p. 176–178 °C; Yield—68 %; IR (v in cm^{-1}): 3119 (NH), 2951 (C–H), 1652 (C=O), 1595 (Ar–C=C), 1520 (asym, Ar–NO_2), 1319 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.48–8.10 (m, 5H, Ar–H and NH), 7.81–7.40 (m, 4H, Ar–H), 5.43–5.32 (m, 2H, CH_2), 4.81 (m, 1H, CH); FAB–MS: m/z 330 ($M+H^+$).

*6-(3,4-Dimethoxyphenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidin-2(1H)-one (**3e**)*

M.p. 204–206 °C; Yield—66 %; IR (v in cm^{-1}): 3313 (NH), 2967 (CH), 1648 (C=O), 1595 (Ar–C=C), 1507 (asym, Ar–NO_2), 1334 (sym, Ar–NO_2), 1262 (–OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.43–8.16 (m, 3H, Ar–H and NH), 7.83–7.80 (d, 2H, Ar–H, $J = 9$ Hz), 6.99–6.85 (m, 3H, Ar–H), 5.21–5.13 (m, 2H, CH_2), 4.59 (m, 1H, CH), 3.78 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3); FAB–MS: m/z 356 ($M+H^+$).

*6-(2,5-Dimethoxyphenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidin-2(1H)-one (**3f**)*

M.p. 230–232 °C; Yield—69 %; IR (v in cm^{-1}): 3174 (NH), 2998 (C–H), 1652 (C=O), 1595 (Ar–C=C), 1505 (asym, Ar–NO_2), 1339 (sym, Ar–NO_2), 1221 (–OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.79 (bs, 1H, NH), 8.19–8.17 (d, 2H, Ar–H, $J = 6.3$ Hz), 7.82–7.79 (d, 2H, Ar–H, $J = 8.4$ Hz), 6.95–6.79 (m, 3H, Ar–H), 4.85–4.79 (m, 2H, CH_2), 4.46 (m, 1H, CH), 3.75 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3); FAB–MS: m/z 356 ($M+H^+$).

*6-(2,4-Dichlorophenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidin-2(1H)-one (**3g**)*

M.p. 190–192 °C; Yield—68 %; IR (v in cm^{-1}): 3252 (NH), 2935 (C–H), 1647 (C=O), 1590 (Ar–C=C), 1507 (asym, Ar–NO_2), 1316 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.43–8.20 (m, 3H, Ar–H and NH), 7.79–7.76 (d, 2H, Ar–H, $J = 9$ Hz), 7.57–7.54 (d, 1H, Ar–H, $J = 8.7$ Hz), 7.43–7.42 (d, 1H, Ar–H, $J = 3.6$ Hz), 7.26 (s, 1H, Ar–H), 5.01–4.92 (m, 2H, CH_2), 4.63 (m, 1H, CH); FAB–MS: m/z 364 ($M+H^+$).

*6-(4-Fluorophenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidin-2(1H)-one (**3h**)*

M.p. 212–214 °C; Yield—74 %; IR (v in cm^{-1}): 3252 (NH), 2929 (C–H), 1669 (C=O), 1594 (Ar–C=C), 1508 (asym, Ar–NO_2), 1311 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.81 (bs, 1H, NH), 8.22–8.18 (d, 2H, Ar–H, $J = 11.4$ Hz), 7.83–7.80 (d, 2H, Ar–H, $J = 8.1$ Hz), 7.74–7.39 (m, 2H, Ar–H), 7.20–7.15 (m, 2H, Ar–H), 5.31–5.18 (m, 2H, CH_2), 4.47 (m, 1H, CH); FAB–MS: m/z 314 ($M+H^+$).

*6-(4-Bromophenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidin-2(1H)-one (**3i**)*

M.p. 178–180 °C; Yield—65 %; IR (v in cm^{-1}): 3210 (NH), 2951 (C–H), 1655 (C=O), 1601 (Ar–C=C), 1514 (asym, Ar–NO_2), 1333 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.62 (s, 1H, NH), 8.39–8.33 (m, 4H, Ar–H), 7.89–7.85 (d, 2H, Ar–H, $J = 10.8$ Hz), 7.69–7.63 (d, 2H, Ar–H, $J = 8.7$ Hz), 5.19–5.11 (m, 2H, CH_2), 4.73 (m, 1H, CH); FAB–MS: m/z 374 ($M+H^+$).

*6-(4-(Dimethylamino)phenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidin-2(1H)-one (**3j**)*

M.p. 220–222 °C; Yield—60 %; IR (v in cm^{-1}): 3148 (NH), 2967 (C–H), 1653 (C=O), 1586 (Ar–C=C), 1516 (asym, Ar–NO_2), 1339 (sym, Ar–NO_2), 1238 (–OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.78 (bs, 1H, NH), 8.32–8.24 (m, 2H, Ar–H), 7.80–7.76 (d, 2H, Ar–H, $J = 12$ Hz), 7.29–7.20 (m, 2H, Ar–H), 6.80–6.68 (m, 2H, Ar–H), 5.15–5.10 (m, 2H, CH_2), 4.49 (m, 1H, CH), 2.93 (s, 6H, $N(CH_3)_2$); FAB–MS: m/z 339 ($M+H^+$).

Synthesis of 6-(substituted phenyl)-5,6-dihydro-4-(4-nitrophenyl)pyrimidine-2(1*H*)-thione (**4a**–**4j** and **10**)

Substituted chalcone (**1a**–**1i**) (20 mmol) and thiourea (20 mmol) were dissolved in ethanol (20 mL) and ethanolic sodium hydroxide (0.2 g in 2.5 mL) was added to reaction mixture. The mixture was refluxed for 8–10 h with constant stirring. The progress of the reaction was monitored through TLC. After completion of reaction, mixture was cooled and poured into ice-cold water with constant stirring for an hour. The mixture was kept in refrigerator for 24 h. The precipitate was filtered, washed with water, dried. The crude product was purified by silica gel column chromatography to afford the corresponding dihydropyrimidine-2(1*H*)-thione derivatives (**4**).

*6-(3-Methoxyphenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidine-2(1H)-thione (**4a**)*

M.p. 158–160 °C; Yield—65 %; IR (v in cm^{-1}): 3243 (NH), 2935 (C–H), 1583 (Ar–C=C), 1514 (asym, Ar–NO_2), 1316 (sym, Ar–NO_2), 1259 (–OCH_3), 1178 (C=S); ^{1}H NMR DMSO (δ in PPM): 9.12 (bs, 1H, NH), 8.43–8.35 (m, 2H, Ar–H), 8.20–8.17 (d, 2H, Ar–H, $J = 8.7$ Hz), 7.56–7.39 (m, 3H, Ar–H), 7.26–7.20 (m, 1H, Ar–H),

5.07–4.99 (m, 2H, CH_2), 4.51 (m, 1H, CH), 3.74 (s, 3H, OCH_3); FAB–MS: *m/z* 342 ($M+H^+$).

*6-(4-Methoxyphenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidine-2(1H)-thione (**4b**)*

M.p. 226–228 °C; Yield—67 %; IR (*v* in cm^{-1}): 3204 (NH), 2933 (C–H), 1595 (Ar–C=C), 1510 (asym, Ar–NO_2), 1332 (sym, Ar–NO_2), 1247 (–OCH_3), 1178 (C=S); ^1H NMR DMSO (δ in PPM): 9.21 (s, 1H, NH), 8.42–8.29 (m, 2H, Ar–H), 8.15–8.11 (m, 2H, Ar–H), 7.79–7.73 (m, 2H, Ar–H), 6.95–6.93 (d, 2H, Ar–H, $J = 6$ Hz), 5.08–5.03 (m, 2H, CH_2), 4.57 (m, 1H, CH), 3.73 (s, 3H, OCH_3); FAB–MS: *m/z* 342 ($M+H^+$).

*6-(4-Chlorophenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidine-2(1H)-thione (**4c**)*

M.p. 204–206 °C; Yield—61 %; IR (*v* in cm^{-1}): 3198 (NH), 3026 (C–H), 1592 (Ar–C=C), 1508 (asym, Ar–NO_2), 1339 (sym, Ar–NO_2), 1181 (C=S); ^1H NMR DMSO (δ in PPM): 9.38 (bs, 1H, NH), 8.32–8.19 (m, 4H, Ar–H), 8.01–7.92 (m, 2H, Ar–H), 7.55–7.52 (m, 2H, Ar–H), 5.14–5.06 (m, 2H, CH_2), 4.67 (m, 1H, CH); FAB–MS: *m/z* 346 ($M+H^+$).

*6-(3-Chlorophenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidine-2(1H)-thione (**4d**)*

M.p. 153–155 °C; Yield—70 %; IR (*v* in cm^{-1}): 3160 (NH), 2969 (C–H), 1591 (Ar–C=C), 1519 (asym, Ar–NO_2), 1331 (sym, Ar–NO_2), 1181 (C=S); ^1H NMR DMSO (δ in PPM): 8.78–8.10 (m, 5H, Ar–H and NH), 7.76–7.41 (m, 4H, Ar–H), 5.23–5.08 (m, 2H, CH_2), 4.79 (m, 1H, CH); FAB–MS: *m/z* 346 ($M+H^+$).

*6-(3,4-Dimethoxyphenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidine-2(1H)-thione (**4e**)*

M.p. 149–151 °C; Yield—72 %; IR (*v* in cm^{-1}): 3368 (NH), 2924 (C–H), 1594 (Ar–C=C), 1509 (asym, Ar–NO_2), 1328 (sym, Ar–NO_2), 1261 (–OCH_3), 1174 (C=S); ^1H NMR DMSO (δ in PPM): 8.73–8.15 (m, 3H, Ar–H, NH), 7.83–7.80 (d, 2H, Ar–H, $J = 8.4$ Hz), 6.99–6.85 (m, 3H, Ar–H), 5.14–5.05 (m, 2H, CH_2), 4.64 (m, 1H, CH), 3.78 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3); FAB–MS: *m/z* 372 ($M+H^+$).

*6-(2,5-Dimethoxyphenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidine-2(1H)-thione (**4f**)*

M.p. 138–140 °C; Yield—65 %; IR (*v* in cm^{-1}): 3240 (NH), 2995 (C–H), 1591 (Ar–C=C), 1524 (asym, Ar–NO_2), 1329 (sym, Ar–NO_2), 1260 (–OCH_3), 1176 (C=S); ^1H NMR DMSO (δ in PPM): 9.05 (bs, 1H, NH), 8.19–8.14 (m, 2H, Ar–H), 7.81–7.79 (d, 2H, Ar–H, $J = 6$ Hz), 6.95–6.79 (m, 3H, Ar–H), 5.11–5.03 (m, 2H, CH_2), 4.71 (m, 1H, CH), 3.77 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3); FAB–MS: *m/z* 372 ($M+H^+$).

*6-(4-Fluorophenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidine-2(1H)-thione (**4g**)*

M.p. 168–170 °C; Yield—74 %; IR (*v* in cm^{-1}): 3234 (NH), 2976 (C–H), 1595 (Ar–C=C), 1507 (asym, Ar–NO_2), 1331 (sym, Ar–NO_2), 1175 (C=S); ^1H NMR DMSO (δ in PPM): 9.41 (bs, 1H, NH), 8.34–8.19 (m, 2H, Ar–H), 7.85–7.79 (d, 2H, Ar–H, $J = 15.6$ Hz), 7.74–7.39 (m, 2H, Ar–H), 7.19–7.14 (m, 2H, Ar–H), 5.17–5.12 (m, 2H, CH_2), 4.74 (m, 1H, CH); FAB–MS: *m/z* 330 ($M+H^+$).

*6-(4-Bromophenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidine-2(1H)-thione (**4h**)*

M.p. 184–186 °C; Yield—69 %; IR (*v* in cm^{-1}): 3184 (NH), 2942 (C–H), 1601 (Ar–C=C), 1509 (asym, Ar–NO_2), 1339 (sym, Ar–NO_2), 1181 (C=S); ^1H NMR DMSO (δ in PPM): 9.42 (s, 1H, NH), 8.49–8.33 (m, 4H, Ar–H), 7.89–7.86 (d, 2H, Ar–H, $J = 9$ Hz), 7.69–7.65 (d, 2H, Ar–H, $J = 11.1$ Hz), 5.18–5.11 (m, 2H, CH_2), 4.73 (m, 1H, CH); FAB–MS: *m/z* 390 ($M+H^+$).

*4-(4-Nitrophenyl)-6-(3,4,5-trimethoxyphenyl)-5,6-dihydropyrimidine-2(1H)-thione (**4i**)*

M.p. 155–157 °C; Yield—67 %; IR (*v* in cm^{-1}): 3217 (NH), 2930 (C–H), 1592 (Ar–C=C), 1511 (asym, Ar–NO_2), 1311 (sym, Ar–NO_2), 1219 (–OCH_3), 1174 (C=S); ^1H NMR DMSO (δ in PPM): 9.21 (s, 1H, NH), 8.24–8.21 (d, 2H, Ar–H, $J = 9$ Hz), 7.87–7.83 (d, 2H, Ar–H, $J = 11.7$ Hz), 6.74 (s, 2H, Ar–H), 5.14–5.06 (m, 2H, CH_2), 4.63 (m, 1H, CH), 3.77 (s, 6H, OCH_3), 3.64 (s, 3H, OCH_3); FAB–MS: *m/z* 402 ($M+H^+$).

*6-(4-(Dimethylamino)phenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidine-2(1H)-thione (**4j**)*

M.p. 193–195 °C; Yield—61 %; IR (*v* in cm^{-1}): 3351 (NH), 2923 (C–H), 1595 (Ar–C=C), 1522 (asym, Ar–NO_2), 1334 (sym, Ar–NO_2), 1170 (C=S); ^1H NMR DMSO (δ in PPM): 8.89 (s, 1H, NH), 8.37–8.31 (m, 2H, Ar–H and CH), 7.79–7.75 (d, 2H, Ar–H, $J = 12.3$ Hz), 7.28–7.21 (m, 2H, Ar–H), 6.78–6.66 (m, 2H, Ar–H), 5.08–5.01 (m, 2H, CH_2), 4.46 (m, 1H, CH), 2.97 (s, 6H, $N(CH_3)_2$); FAB–MS: *m/z* 355 ($M+H^+$).

General synthesis of 5-(substituted phenyl)-3-(4-nitrophenyl)isoxazole (**5a**–**5f**)

Substituted chalcone (**1a**–**1k**) (10 mmol) and hydroxylamine hydrochloride (10 mmol) were taken in ethanol (10 mL). 50 % ethanolic potassium hydroxide solution (2 mL) was added drop wise to the reaction mixture with stirring at rt. The reaction mixture was refluxed for 10–18 h. The progress of the reaction was monitored through TLC. After completion of reaction, mixture was cooled and acidified with glacial acetic acid. The excess solvent was removed under reduced pressure and concentrate was poured into ice water. The precipitate was filtered, washed with water, dried. The crude product was purified by silica gel column chromatography to afford the corresponding isoxazole derivatives (**5**).

*5-(3-Methoxyphenyl)-3-(4-nitrophenyl)-4,5-dihydroisoxazole (**5a**)*

M.p. 174–176 °C; Yield—72 %; IR (ν in cm^{-1}): 3032 (CH), 1609 (Ar–C=C), 1520 (asym, Ar–NO_2), 1349 (sym, Ar–NO_2), 1244 (OCH_3);^{1}H NMR DMSO (δ in PPM): 8.38–8.31 (m, 4H, Ar–H), 7.92–7.87 (d, 1H, Ar–H, $J = 13.8$ Hz), 7.53–7.39 (m, 2H, Ar–H), 6.96–6.93 (d, 1H, Ar–H, $J = 9$ Hz), 5.80–5.74 (dd, 1H, CH, $J = 7.9$, 11.7 Hz), 3.97–3.89 (dd, 1H, CH_2, $J = 10.9$, 17.3 Hz), 3.76 (s, 3H, OCH_3), 3.53–3.45 (dd, 1H, CH_2, $J = 8.1$, 15.9 Hz); FAB–MS: *m/z* 299 ($M+H^+$).

*5-(4-Chlorophenyl)-3-(4-nitrophenyl)-4,5-dihydroisoxazole (**5b**)*

M.p. 122–124 °C; Yield—74 %; IR (ν in cm^{-1}): 3034 (CH), 1612 (Ar–C=C), 1511 (asym, Ar–NO_2), 1335 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.37–8.32 (m, 4H, Ar–H), 7.77–7.71 (m, 2H, Ar–H), 7.31–7.27 (m, 2H, Ar–H), 5.75–5.71 (dd, 1H, CH, $J = 6.2$, 7.5 Hz), 3.96–3.89 (dd, 1H, CH_2, $J = 8.3$, 15.5 Hz), 3.54–3.45 (dd, 1H, CH_2, $J = 8.4$, 16.1 Hz); FAB–MS: *m/z* 303 ($M+H^+$).

*5-(3-Chlorophenyl)-3-(4-nitrophenyl)-4,5-dihydroisoxazole (**5c**)*

M.p. 138–140 °C; Yield—70 %; IR (ν in cm^{-1}): 3023 (CH), 1595 (Ar–C=C), 1516 (asym, Ar–NO_2), 1319 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.37–8.32 (m, 4H, Ar–H), 7.56–7.24 (m, 4H, Ar–H), 5.81–5.73 (dd, 1H, CH, $J = 9$, 15 Hz), 3.98–3.89 (dd, 1H, CH_2, $J = 10.8$, 16.2 Hz), 3.55–3.48 (dd, 1H, CH_2, $J = 8.2$, 14.3 Hz); FAB–MS: *m/z* 303 ($M+H^+$).

*5-(3,4-Dimethoxyphenyl)-3-(4-nitrophenyl)-4,5-dihydroisoxazole (**5d**)*

M.p. 106–108 °C; Yield—75 %; IR (ν in cm^{-1}): 2924 (CH), 1594 (Ar–C=C), 1507 (asym, Ar–NO_2), 1306 (sym, Ar–NO_2), 1259 (OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.37–8.32 (m, 4H, Ar–H), 7.15–6.93 (m, 3H, Ar–H), 5.78–5.73 (dd, 1H, CH, $J = 6$, 9.9 Hz), 3.98–3.89 (dd, 1H, CH_2, $J = 11.2$, 15.6 Hz), 3.76 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.52–3.43 (dd, 1H, CH_2, $J = 8.2$, 18.1 Hz); FAB–MS: *m/z* 329 ($M+H^+$).

*5-(2,4-Dichlorophenyl)-3-(4-nitrophenyl)-4,5-dihydroisoxazole (**5e**)*

M.p. 160–162 °C; Yield—67 %; IR (ν in cm^{-1}): 3012 (CH), 1590 (Ar–C=C), 1520 (asym, Ar–NO_2), 1344 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.29–8.25 (d, 2H, Ar–H, $J = 11.6$ Hz), 7.99–7.93 (m, 2H, Ar–H), 7.54–7.51 (d, 1H, Ar–H, $J = 9.3$ Hz), 7.41–7.36 (d, 1H, Ar–H, $J = 14.1$ Hz), 7.24 (s, 1H, Ar–H), 5.86–5.79 (dd, 1H, CH, $J = 8.2$, 12.7 Hz), 3.96–3.88 (dd, 1H, CH_2, $J = 10.5$, 13.8 Hz), 3.53–3.45 (dd, 1H, CH_2, $J = 9$, 15.6 Hz); FAB–MS: *m/z* 337 ($M+H^+$).

*5-(4-Fluorophenyl)-3-(4-nitrophenyl)-4,5-dihydroisoxazole (**5f**)*

M.p. 157–159 °C; Yield—64 %; IR (ν in cm^{-1}): 2921 (CH), 1603 (Ar–C=C), 1508 (asym, Ar–NO_2), 1342 (sym, Ar–NO_2), 1225 (Ar–F); ^{1}H NMR DMSO (δ in PPM): 8.44–8.41 (d, 2H, Ar–H, $J = 8.1$ Hz), 8.20–8.17 (d, 2H, Ar–H, $J = 8.4$ Hz), 8.02–7.96 (m, 2H, Ar–H), 7.60–7.39 (m, 2H, Ar–H), 5.88–5.81 (dd, 1H, CH, $J = 8.2$, 11.8 Hz), 3.99–3.89 (dd, 1H, CH_2, $J = 10.9$, 17.3 Hz), 3.54–3.45 (dd, 1H, CH_2, $J = 9$, 17.3 Hz); FAB–MS: *m/z* 287 ($M+H^+$).

Evaluation of antimycobacterial activity

Determination of minimum inhibitory concentration (MIC)

The synthesized compounds after confirmation of their structures were subjected to biological evaluation. The MIC was determined by agar dilution method. The procedure followed for anti-TB activity mainly involves the use of Middlebrook 7H11 agar with OADC growth supplement and standard strain of *M. tuberculosis* $H_{37}Rv$ (ATCC 27294). The basal medium is prepared and sterilized by autoclaving. The stock solution of the test

compounds and standard drugs (isoniazid, rifampicin, and ethambutol) was prepared in DMSO. Twofold serial dilutions of each test compound/drug were incorporated into Middlebrook 7H11 agar medium with OADC growth supplement. Inoculum of *M. tuberculosis* $H_{37}Rv$ were prepared from fresh Middlebrook 7H11 agar slants with OADC growth supplement adjusted to 1 mg mL^{-1} in Tween 80 (0.05 %) saline diluted to 10^{-2} to give a concentration of approximately 10^7 cfu mL^{-1}. The tubes were incubated at 37 °C. Along with this one growth control without compound and drug controls were also set up. The tubes are inspected for growth twice a week for a period of 3 weeks and final readings were recorded after 28 days.

QSAR study

In an attempt to determine the role of structural features which appears to influence the observed activity of chalcone and their substituted nitroaryl derivatives (**1a–1i**, **2a–2j**, **3a–3j**, **4a–4j** and **5a–5f**), QSAR studies were performed using SQ-MLR model. The pMIC value of the biological activity data (excluding six compounds, which MIC value numerically not well defined) was used as dependent variable in QSAR study. The dataset was divided into training set (30 compounds; TR-1 to TR-30) and test set (9 compounds; TE-1 to TE-9). These were correlated with different molecular descriptors like constitutional, topological, geometrical, charge, GETAWAY (Geometry, Topology and Atoms-Weighted AssemblY), WHIM (Weighted Holistic Invariant Molecular descriptors), 3D-MoRSE (3D-Molecular Representation of Structure based on Electron diffraction), molecular walk counts, BCUT descriptors, 2D-Autocorrelations, aromaticity indices, Randic molecular profiles, radial distribution functions.

Molecular modeling study was carried out through *CS ChemOffice* (CS ChemOffice, 6.0, 2000), *DRAGON* (Todeschini and Consonni, 2001), and *VALSTAT* (Gupta *et al.*, 2004) software. The structure of substituted nitroaryl analogs were subjected to energy minimization using molecular mechanics (MM2) until the RMS gradient value became smaller than 0.419 kJ mol^{-1} $Å^{-1}$. The energy minimized molecules were subjected to re-optimization via AM1 method until the RMS gradient attains a value smaller than 0.419 J mol^{-1} $Å^{-1}$ using MOPAC. The geometry optimization of the lowest energy structure was carried out using EF routine. The QSAR descriptors of substituted nitroaryl analogs were calculated using the molecular package *DRAGON*. Further, the regression analysis was performed using the statistical program *VALSTAT*.

The statistical fitness of the regression equations were assessed by parameters like Pearson correlation coefficient (r), coefficient of determination (r^2), explained variance (r^2_{adj}), standard error of estimate (SEE), and sequential Fischer test (F) at specified degree of freedom (df). The quality of the regression equations were analyzed by parameters such as quality factor (QF), pair-wise correlation (PWC), variance inflation factor (VIF), probable error of coefficient of determination (PE), outlier (Z_{value}), Akaike's information criterion (AIC) and Kubinyi function (FIT).

The robustness of the regression equation was ascertained through bootstrapping analysis and response scrambling analysis (Y-randomization test) techniques. Bootstrapping squared correlation coefficient (r^2_{BS}) is the average squared correlation coefficient of subset of compounds used in regression, while bootstrapping standard deviation ($r^2_{BS\_STD}$) is the standard deviation of n run data of bootstrapping method. Response scrambling analysis was evaluated by parameters like randomized squared correlation coefficient ($r^2_{RAND\_MAX}$), mean randomized squared correlation coefficient ($r^2_{RAND\_MEAN}$), standard deviation of randomized squared correlation coefficient ($r^2_{RAND\_STD}$) and chance correlation factor (Chance).

Result and discussion

Chemistry

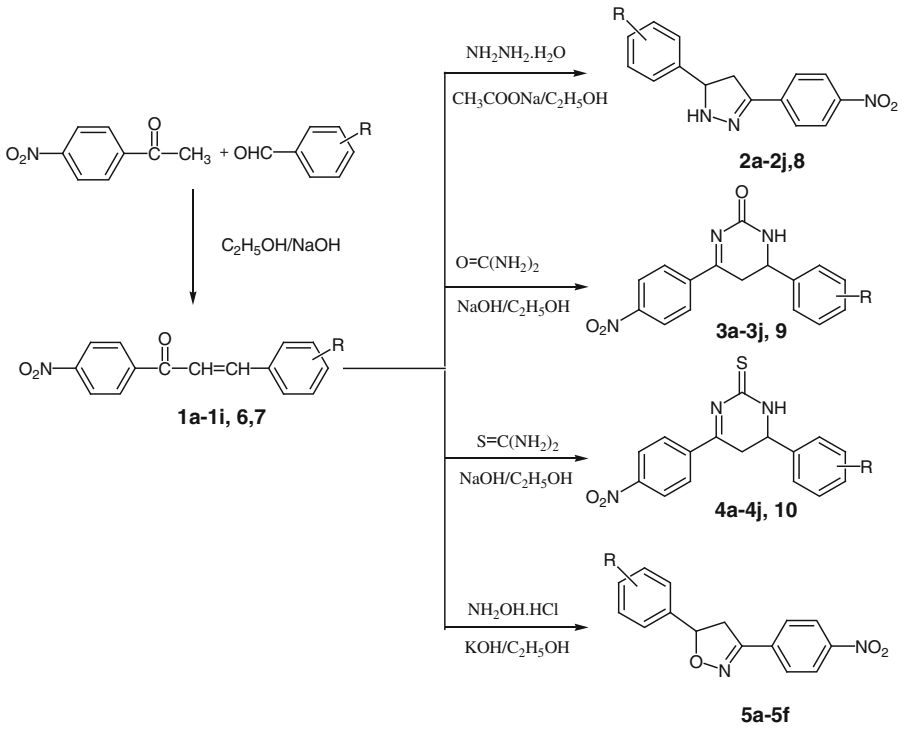
In this study, a series of 45 new compounds were synthesized. Scheme 1 illustrates the synthetic route for the preparation of target compounds. Chalcones are synthesized by Claisen–Schmidt condensation. In Claisen–Schmidt condensation, an aromatic aldehyde react with an aryl methyl ketones/methyl ketones in the presence of a base to form an α,β-unsaturated ketone (enone). In the initial step, chalcones were synthesized by condensing 4-nitroacetophenone with appropriate aromatic aldehydes in ethanolic sodium hydroxide solution at room temperature (**1a–1i**). From the chalcone derivatives, the various heterocyclic derivatives like pyrazole (**2a–2j**), oxopyrimidine (**3a–3j**), thiopyrimidine (**4a–4j**), and isoxazole (**5a–5f**) were synthesized via cyclocondensation with hydrazine hydrate, urea, thiourea, and hydroxylamine hydrochloride, respectively. The purity of the compounds was checked via TLC using various mobile bases and the compounds of this study were identified by spectral data.

Both analytical and spectral data (^{1}H NMR, IR spectroscopy, Mass Spectroscopy) of all the synthesized compounds were in full agreement with the proposed structures. In the ^{1}H NMR spectra, the signals of the respective protons of the prepared derivatives were verified on the basis of their chemical shifts and multiplicities.

Antimycobacterial activity

The synthesized compounds were screened for their antimycobacterial activity in vitro against *M. tuberculosis*

Scheme 1 General synthetic scheme of nitroaryl derivatives



(*MTB*) *H*$_{37}$*Rv* by agar dilution method. The MIC is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth. MICs of the synthesized compounds along with the standard drugs for comparison are reported in Table 1.

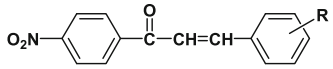
Among the chalcones, 3-(2,4-dichlorophenyl)-1-(4-nitro phenyl)prop-2-en-1-one (**1g**) and 3-(4-fluorophenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**1h**) shows MIC value 1.56 and 3.13 μg mL^{-1}, respectively. Chalcone analogs having 3-methoxy (**1a**), 4-methoxy (**1b**), 3-chloro (**1d**), 4-chloro (**1c**), 2,5-dimethoxy (**1f**), 3,4-dimethoxy (**1e**) and 3,4,5-trimethoxy (**1i**) substitution shows MIC value more than or equal to 12.5 μg mL^{-1}. In pyrazole analogs, compounds **2g** and **2h** shows MIC value 12.5 μg mL^{-1}, whereas the rest of the analogs shows MIC value more than or equal to 25.0 μg mL^{-1}. 6-(4-fluorophenyl)-4-(4-nitrophenyl)-5, 6-dihydropyrimidin-2(1*H*)-one (**3h**) produced inhibition at 6.25 μg mL^{-1} as compared to thiopyrimidines (**4g**) exhibited inhibition at 12.5 μg mL^{-1}. On the other hand, the analog of substituted methoxy, 2,4-dichloro, bromo and *p*-dimethylamino shows relatively moderate-to-low antimycobacterial activity.

Preliminary structure activity relationship analysis revealed that chalcone analogs are more potent in comparison to substituted pyrazole, isoxazole, pyrimidin-2(1*H*)-one, and pyrimidine-2(1*H*)-thione derivatives. Substitution of electron withdrawing groups (i.e., –F, –Cl, –Br) on phenyl ring favorable for the activity, while electron releasing groups (i.e., –OCH_3, –$N(CH_3)_2$) diminish the activity. Di-substituted halogen containing compounds are more potent as compare to mono-substituted halogen compounds. 3-methoxy, 4-methoxy, 2,5-dimethoxy, 3,4-dimethoxy, 3,4,5-trimetoxy and *N,N*-dimethylamino derivatives are comparatively less potent than 4-flouro, 3-chloro, 4-chloro, 4-bromo and 2,4-dichloro derivatives of chalcone, pyrazole, isoxazole, pyrimidin-2(1*H*)-one, and pyrimidine-2(1*H*)-thione.

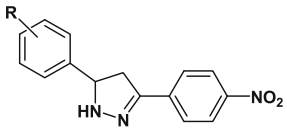
Model and discussions

Quantitative models were developed by means of structural contribution considering regression methodology. The multivariant expressions were developed on the basis of adjustable correlation coefficient (r^2_{adj}) (Gupta *et al.*, 2008). This parameter explains statistical significance of incorporated physicochemical descriptors in SEQ-MLR. r^2_{adj} takes into account of adjustment of coefficient of determination. If r^2_{adj} value decline by the addition of a physicochemical descriptor to the equation it is indicated that descriptor was not contributed fairly. Adjustable correlation coefficient is a measure of % explained variation of regression expression, whereas r^2 always increases when an independent variable is added. Study has furnished uni and bi-variant expression with moderate correlation coefficient Eqs. (1)

Table 1 Structure and Anti-mycobacterial activity data of nitroaryl analogs



Comp. No. 1a-1i



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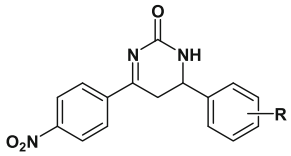

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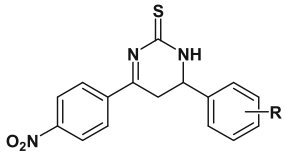
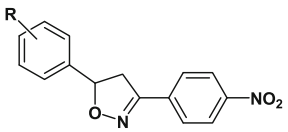


Comp. No. 4a-4j



Comp. No. 5a-5f

Comp. no	R	MIC (μg mL^{-1})[a]	MIC (μM)[b]	pMIC[c]
1a	3-OCH_3	25	88.34	4.054
1b	4-OCH_3	>25.0	>88.34	–
1c	4-Cl	12.5	43.55	4.362
1d	3-Cl	12.5	43.55	4.362
1e	3,4-OCH_3	>25.0	>79.87	–
1f	2,5-OCH_3	25	79.87	4.098
1g	2,4-Cl	1.56	4.84	5.315
1h	4-F	3.13	11.55	4.938
1i	3,4,5-OCH_3	25	72.89	4.138
2a	3-OCH_3	100	338.98	3.470
2b	4-OCH_3	>100	>338.98	–
2c	4-Cl	25	83.61	4.079
2d	3-Cl	25	83.61	4.079
2e	3,4-OCH_3	50	153.85	3.813
2f	2,5-OCH_3	50	153.85	3.813
2g	2,4-Cl	12.5	37.43	4.427
2h	4-F	12.5	44.17	4.355
2i	3,4,5-OCH_3	50	140.85	3.852
2j	4-Br	25	72.67	4.139
3a	3-OCH_3	100	307.69	3.510
3b	4-OCH_3	50	153.85	3.811
3c	4-Cl	12.5	37.99	4.419
3d	3-Cl	12.5	37.99	4.419
3e	3,4-OCH_3	100	281.69	3.548
3f	2,5-OCH_3	>100	>281.69	–
3g	2,4-Cl	12.5	34.34	4.462
3h	4-F	6.25	19.97	4.697
3i	4-Br	12.5	33.42	4.474
3j	4-$N(CH_3)_2$	100	295.86	3.527
4a	3-OCH_3	100	293.26	3.531
4b	4-OCH_3	100	293.26	3.531
4c	4-Cl	12.5	36.23	4.439
4d	3-Cl	25	72.46	4.138
4e	3,4-OCH_3	50	134.77	3.869
4f	2,5-OCH_3	50	134.77	3.869
4g	4-F	12.5	37.99	4.418
4h	4-Br	12.5	32.05	4.492
4i	3,4,5-OCH_3	100	249.38	3.601
4j	4-$N(CH_3)_2$	50	141.24	3.848
5a	3-OCH_3	>50	>168.91	–
5b	4-Cl	25	83.33	4.080
5c	3-Cl	>25	>83.33	–
5d	3,4-OCH_3	25	76.69	4.116
5e	2,4-Cl	12.5	37.31	4.428
5f	4-F	12.5	44.01	4.357
Isoniazid	–	0.049	0.357	6.447
Rifampicin	–	0.098	0.119	6.924
Ethambutol	–	1.56	7.635	5.117

[a] Minimum inhibitory concentration against *M. tuberculosis* in μg/mL

[b] Minimum inhibitory concentration in μM

[c] Negative logarithmic minimum inhibitory concentration in mole

and (2) respectively but the r^2_{adj} value is increasing significantly from uni to bi-variant expression.

$$\begin{aligned} \text{pMIC} &= -2.164(\pm 0.313)\text{BELv5} + 6.763 \\ & n = 30,\ r = 0.794,\ r^2_{adj} = 0.617, \\ & \text{SEE} = 0.257,\ F = 47.698 \end{aligned} \quad (1)$$

$$\begin{aligned} &\text{pMIC} = 1.943(\pm 0.275)\text{Ms} + 6.623(\pm 0.973)\text{ATS8v} - 3.922 \\ &n = 30,\ r = 0.897,\ r^2_{adj} = 0.791,\ \text{SEE} = 0.2190, \\ &F = 55.854 \end{aligned} \quad (2)$$

Significant improvement in r^2_{adj} value emphasizes to explore the higher variant expressions like tri-variant expressions. Proposed model should have to satisfy both statistical quality and predictive power. Therefore, all the developed tri-variant expressions were validated through leave-*n*-out cross-validation method, bootstrapping technique, and response scrambling analysis test. Equation (3) which fulfills all the corroboration criteria up to significant echelon was considered as best QSAR model for *M. tuberculosis*.

$$\begin{aligned} \text{pMIC} &= 3.979(\pm 0.332)\text{Ms} + 14.607(\pm 1.838)^0_{\chi\text{Av}} \\ &\quad - 0.399(\pm 0.083)^3_s - 13.004 \\ & n = 30,\ r = 0.921,\ r^2_{adj} = 0.831, \\ & \text{SEE} = 0.171,\ F = 48.392 \end{aligned} \quad (3)$$

Table 2 Pair wise correlation (PWC) and variance inflation factor (VIF) values of the descriptors used in QSAR model

Equation	Descriptors	VIF	PWC		
			Ms	$^{0}\chi_{Av}$	$^{3}\kappa_{s}$
3	*Ms*	1.836	1.000		
	$^{0}\chi_{Av}$	2.149	0.630	1.000	
	$^{3}\kappa_{s}$	1.295	0.019	0.382	1.000

Quality parameters : QF = 5.385, PE = 0.018, FIT = 3.722, LOF = 1.294, AIC = 0.038, PWC < 0.635, VIF < 2.220, Outlier = Nil.

Validation parameters : $r^2_{BS} = 0.855, r^2_{BS\_STD} = 0.081$, Chance < 0.001, $r^2_{RAND\_MAX} = 0.452$, $r^2_{RAND\_MEAN} = 0.104$, $r^2_{RAND\_STD} = 0.077, {}_1Q^2 = 0.797$, ${}_1S_{PRESS} = 0.198, {}_1S_{DEP} = 0.184, {}_{10}Q^2 = 0.771$, ${}_{10}S_{PRESS} = 0.197, {}_{10}S_{DEP} = 0.196, r^2_{PRED(n=9)} = 0.672$.

The statistically best model (Eq. 3) for anti-mycobacterial activity against *M. tuberculosis* with a coefficient of determination ($r^2 = 0.848$), which accounts for more than 83.1 % of the explain variance in the activity was considered for further study.

A high correlation coefficient alone is not enough to select the equation as a model; hence various statistical approaches were employed to confirm the robustness and the practical applicability of the equations. Equations were screened through various internal and external statistical validation techniques. Internal statistical significance level of the equations was confirmed using sequential Fischer test. Equation (3) shows significance level more than 99.9 % as it exceeded the tabulated $F_{(3,26,\ \alpha\ 0.001)} = 8.269$. High values of the *F* indicate that the model is statistically significant. The equation was further tested for outlier by the *Z*-score method (Gupta *et al.*, 2010) and no compound was found to be an outlier, suggesting that the equation is able to explain the structurally diverse analogs of the series.

The interdependency of physicochemical properties for each equation was checked by pair wise correlation analysis (PWC) to confirm inimitable contribution of the properties to the expression (Table 2). From the correlation matrix, it was observed pair wise correlation values are ≤0.630 between the descriptors suggested that independent contribution. To further check the multi-colinearity problem between the descriptors variance inflation factor (VIF) (Chatterjee *et al.*, 2000; Shapiro and Guggenheim, 1998) analysis was performed (Table 2). VIF values for the model is less than 2, which is much lesser than critical value 10, indicating that these models reach the statistical requirements and that there is no co-linearity problem.

It is necessary that the selected equations should have both the statistical quality as well as better predictive power. The aforementioned discussion and the regression parameters are good enough to establish the quality of model. However, the simplest parameter to decide the predictive power of the model is the quality factor QF (Pogliani, 1994, 1996). Selected QSAR equation has good statistical quality with significant value of QF which indicates best predictive power of the model.

Attempt was also made to investigate goodness of fit for the model using statistical parameter called probable error of correlation (PE) (Mandloi *et al.*, 2005). Selected QSAR equation has multiple regression coefficient (*r*) value much greater than 6PE. Thus, the correlation attempted is definitely good.

Additional statistical parameters, such as the Akaike's information criterion (AIC) (Akaike, 1973, 1974) the Kubinyi function (FIT) (Kubinyi, 1994a, b) and the Friedman's lack of fit (LOF) (Friedman, 1990), have also been calculated to further validate the equation. The model that produces the lowest AIC value and highest FIT value is considered potentially the most useful and the best. The LOF factor takes into account the number of terms used in the equation and is not biased, as are other indicators, toward large number of parameters. The decreased values of parameters AIC and LOF and increased value of FIT have further shown the superiority of this model.

The value of bootstrapping squared correlation coefficient (r^2_{BS}) and the bootstrapping standard deviation implies that the equations were proper representatives of the group of analogs. The r^2_{BS} is at par with the coefficient of determination (r^2) with low value of bootstrapping standard error suggested the robustness of the model. The value of chance correlation factor (Chance <0.001) revealed that the results were not based on prospective correlation. Similarly, max randomized r^2 ($r^2_{RAND\_MAX}$), mean randomized r^2 ($r_{RAND\_MEAN}{}^2$) and randomized standard deviation ($r^2_{RAND\_STD}$) values are also supporting that the results are not based on chance correlation. The internal consistency of the training set was confirmed through leave-*n*-out cross-validation method. Model showed good internal consistency in leave-one-out and leave-ten-out techniques (${}_1Q^2 = 0.797$ and ${}_{10}Q^2 = 0.771$). Leave-one-out (${}_1Q^2$) and leave-ten-out (${}_{10}Q^2$) values found to be significantly more than 0.5, which minimizes the risk of finding a significant explanatory equation for the biological activity just by mere chance. These may not be applicable for the analogs, which were never used in the generation of the correlation. Therefore, predictive power of selected model was further confirmed by a test set of nine compounds. The robustness and wide applicability of the model was further explained by a significant $r^2_{pred\ (n=9)}$ value (0.672) of the test set data. In order to confirm the results, the comparison of experimental

Table 3 Experimental, calculated, calculated (leave-one-out), residual and *Z* score data of nitroaryl analogs training set compounds

Comp. no	Training (TR) set	pMIC					
		Exp[a]	Cal[b]	Res^{c}_{cal}	Z_{value}	$Cal^{d}_{(LOO)}$	$Res^{e}_{Cal(LOO)}$
1a	TR-1	4.054	4.117	−0.063	−0.386	4.124	−0.070
1c	TR-2	4.362	4.575	−0.213	−1.315	4.601	−0.239
1f	TR-3	4.098	4.138	−0.040	−0.247	4.145	−0.047
1g	TR-4	5.315	5.071	0.244	1.505	4.965	0.350
1h	TR-5	4.938	4.886	0.052	0.322	4.865	0.073
2c	TR-6	4.079	4.019	0.060	0.368	4.012	0.067
2d	TR-7	4.079	4.019	0.060	0.368	4.012	0.067
2a	TR-8	3.470	3.583	−0.113	−0.695	3.606	−0.136
2h	TR-9	4.355	4.303	0.052	0.324	4.293	0.063
2j	TR-10	4.139	4.320	−0.181	−1.120	4.369	−0.230
2e	TR-11	3.813	3.663	0.151	0.931	3.645	0.169
2g	TR-12	4.427	4.513	−0.086	−0.528	4.525	−0.098
3b	TR-13	3.811	3.877	−0.067	−0.412	3.885	−0.074
3d	TR-14	4.419	4.324	0.095	0.585	4.319	0.100
3c	TR-15	4.419	4.324	0.095	0.585	4.319	0.100
3h	TR-16	4.697	4.574	0.123	0.760	4.546	0.151
3i	TR-17	4.474	4.579	−0.106	−0.652	4.592	−0.118
3e	TR-18	3.548	3.919	−0.371	−2.291	3.959	−0.410
4b	TR-19	3.531	3.750	−0.219	−1.351	3.769	−0.238
4h	TR-20	4.492	4.440	0.052	0.321	4.423	0.069
4d	TR-21	4.138	4.185	−0.047	−0.288	4.188	−0.050
4e	TR-22	3.869	3.789	0.080	0.494	3.779	0.090
4c	TR-23	4.439	4.185	0.254	1.571	4.169	0.271
4f	TR-24	3.869	3.789	0.080	0.494	3.779	0.090
4j	TR-25	3.848	3.617	0.232	1.430	3.575	0.273
4i	TR-26	3.601	3.772	−0.170	−1.052	3.809	−0.208
5d	TR-27	4.116	3.763	0.352	2.176	3.731	0.385
5b	TR-28	4.080	4.145	−0.065	−0.401	4.152	−0.072
5e	TR-29	4.428	4.599	−0.170	−1.052	4.623	−0.194
5f	TR-30	4.357	4.429	−0.072	−0.445	4.446	−0.089

[a] Experimental data of the compounds used in generation of model

[b] Calculated data of the compounds using model

[c] Residual value of calculated data

[d] Calculated leave-one-out data of the compounds using model

[e] Residual value of calculated leave-one-out data

Table 4 Experimental, predicted and residual values of nitroaryl analogs test set compounds

Comp. no	Test (TE) set	pMIC		
		Exp[a]	Pred[b]	Res^{c}_{pred}
1d	TE-1	4.362	4.575	−0.213
1i	TE-2	4.138	4.124	0.013
2f	TE-3	3.813	3.663	0.151
2i	TE-4	3.852	3.673	0.179
3a	TE-5	3.510	3.877	−0.368
3g	TE-6	4.462	4.725	−0.263
3j	TE-7	3.527	3.774	−0.248
4g	TE-8	4.418	4.437	−0.019
4a	TE-9	3.531	3.750	−0.219

[a] Experimental data of the test set compounds

[b] Predicted data of the test set compounds

[c] Residual value of test set compounds

and predicted (LOO) and predicted data (Tables 3, 4) demonstrated that they are close to each other evidenced by the low residual activity values. Further their fitness and internal predictability was supported by the plot of experimental pMIC against calculated pMIC and experimental pMIC against predicted (LOO) pMIC (Figs. 1, 2) respectively, while external predictivity was supported by the plot of experimental pMIC against predicted pMIC (Fig. 3). In general, the selected model fulfills the statistical validation criteria to a significant extent.

QSAR analysis revealed that molecular descriptors mean electrotopological state (Ms) and average valance connectivity index chi-0 ($^{0}\chi_{Av}$) contributed positively, while 3-path Kier alpha-modified shape index ($^{3}\kappa_{\alpha}$) contributed negatively to the anti-mycobacterial activity of the analogs.

The mean electrotopological state (Ms) is derived from Kier–Hall electrotopological state index, (Kier and Hall,

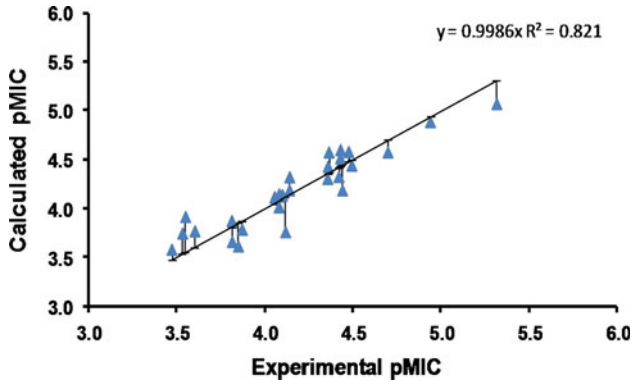


Fig. 1 A *plot* of experimental and calculated pMIC of training set compounds using selected model

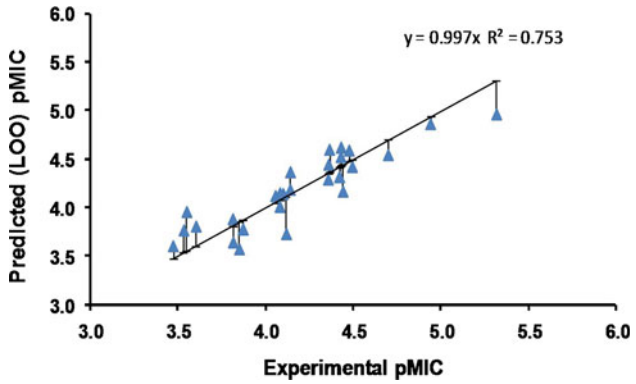


Fig. 2 A *plot* of experimental and predicted (leave-one-out) pMIC of training set compounds using selected model

1990, 1999) and its gives information related to the electronic and topological state of the atom in the molecule. The mean electrotopological state (Ms) is calculated by dividing Kier–Hall electrotopological state (Ss) by the number of nonhydrogen atoms (*n*SK).

$$\mathrm{Ms} = \mathrm{Ss}/n\mathrm{SK}$$

Ss depend on intrinsic state of the *i*th atom and field effect on the *i*th atom calculated as perturbation of the intrinsic state of the *i*th atom by all other atoms in the molecule. The intrinsic state of an atom is the ratio of π and lone pair electrons to the count of the σ bonds in the molecular graph for the considered atom. Therefore, the intrinsic state reflects the possible partitioning of non-σ electrons influence along the paths starting from the considered atom; the less partitioning of the electron influence, the more available are the valence electrons for intermolecular interactions. Large positive values of Ss relate to atoms of high electronegativity and/or terminal atoms or atoms that lie on the mantle of the molecule; small or negative Ss values correspond to atoms possessing only σ electrons and/or buried in the interior of the molecule or close to higher electronegative atoms. Therefore, the Ss is a

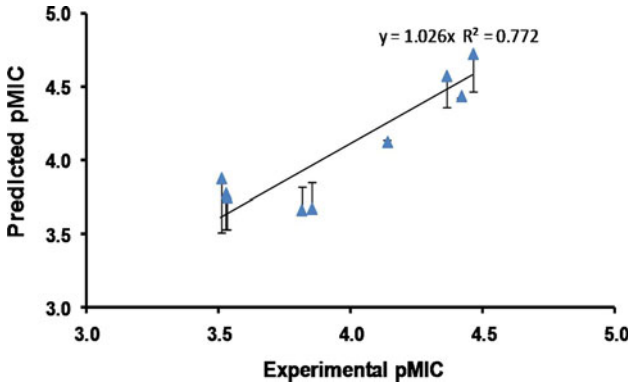


Fig. 3 A *plot* of experimental and predicted pMIC of test set compounds using selected model

measure of the electronic accessibility of an atom and can be interpreted as a probability of interaction with another molecule. However, the index cannot be considered a pure electronic descriptor: it is, in fact, a descriptor of atom polarity and steric accessibility. This corresponds to an electronegativity equalization principle and means that the sum of the electrotopological states in the molecule depends on only the number and type of atoms, not on their mutual interactions.

Positive contribution of mean electrotopological state (Ms) revealed that electronic interactions with macromolecule are crucial for the activity and electron withdrawing groups are favor for the anti-mycobacterial activity.

Intermolecular encounters of molecules, governed by the pattern of atoms, and bonds, these structure features are expected to have a relationship to activity arising from intermolecular interactions and may also function to provide discrimination among multiple structural classes. Second, atom-type and bond-type descriptors encode information on specific molecule features such as atom and bond types associated with important functional groups. Molecular connectivity is a method of molecular structure quantitation in which weighted counts of substructure fragments are incorporated into numerical indices. Structural features such as size, branching, unsaturation, heteroatom content, and cyclicity are encoded. ${}^{0}\chi_{\mathrm{Av}}$ is average valence connectivity index chi-0 (${}^{0}\chi$) (Kier, 1980; Kier and Hall, 1983, 1986). ${}^{0}\chi_{\mathrm{Av}}$ mathematically calculated as

$$m_{\chi v} = \sum_{i=1}^{N_S} \prod_{k=1}^{m+1} \left(\frac{1}{\delta_k^v}\right)^{1/2} \quad \text{where } \delta_k^v = \frac{(Z_k^v - H_k)}{(Z_k - Z_k^v - 1)}$$

δ_k^v is valence connectivity for the *k*th atom in the molecular graph; N_s is the number of non-hydrogen atoms in the molecule; Z_k is the total number of electrons in the *k*th atom; Z_k^v is the number of valence electrons in the *k*th atom; H_k is the number of hydrogen atoms directly attached to the *k*th non-hydrogen atom; *m* is 0 for atomic valence connectivity indices. The 0th order χ index holds little structural information. Only the presence of the nearest

neighbor to each atom is captured. However, δ^v distinguishes between multiple and single bonds. It's also distinction between element rows.

χ index encode degree of skeletal branching and molecular size. Low order indices $^0\chi$ and $^1\chi$ increase with molecular size and decrease with increased branching. Increase in $^0\chi$, which reflects the increasing size of the molecule skeleton. $^0\chi_v$ index is highly inter-correlated with molecular surface area and volume. $^0\chi_{Av}$ is average of the $^0\chi_v$ index, positive contribution of this descriptor revealed that bulkier substitutions favor for the activity. These groups might be helpful in favorable enthalpic interactions.

Kier alpha-modified shape ($^3\kappa_\alpha$) (Kier, 1986) descriptor accounts the different shape contribution of heteroatoms and hybridization states of the molecules. It is a function of the number of atoms and their bonding relationship. The negative contribution revealed that branched molecule favor for the activity in comparison with non-branching or when it is located at the extremities of a graph.

Designed compounds

On the basis of contributing parameters, newer analogs were designed and the activity was predicted through model. Structure of designed compounds (**6–10**) and their predicted activity are shown in Table 5. The required absorption characteristic of the designed compound was checked to confirm their bioavailability using Lipinski rule (Table 6). The designed compounds were synthesized by above-mentioned methods. Anti-mycobacterial activity of the proposed synthesized compounds was determined using agar dilution method. The experimental and model predicted pMIC of these compounds (**6–10**) are shown in Table 5 and Fig. 4. The experimental activity data of the proposed compounds was found to be comparable with that of the predicted activity using model. This data suggested that the QSAR models could be further used for designing of new analogs.

*3-(4-Bromophenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**6**)*

M.p. 152–154 °C; Yield—75 %; IR (v in cm^{-1}): 1658 (C=O), 1603, 1487 (Ar–C=C), 1514 (asym, Ar–NO_2), 1318 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.39–8.33 (m, 4H, Ar–H), 8.01–7.97 (d, 1H, CH=CH, $J = 11.6$ Hz), 7.89–7.87 (d, 2H, Ar–H, $J = 6$ Hz), 7.69–7.67 (d, 2H, Ar–H, $J = 6$ Hz), 7.53–7.48 (d, 1H, CH=CH, $J = 14.6$ Hz); FAB–MS: m/z 332 ($M+H^+$).

Table 5 Structure, predicted pMIC and experimental MIC and pMIC of proposed synthesized compound

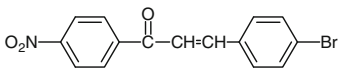
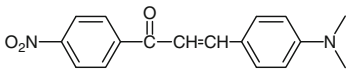
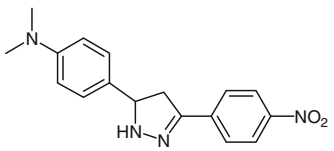
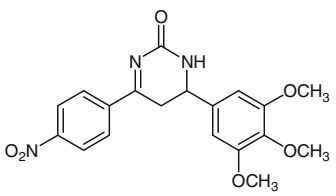
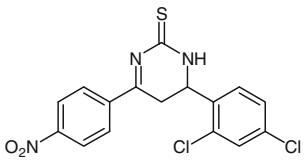
Comp. no	Structure	MIC		pMIC	
		μg mL^{-1}	μM	Experimental	Predicted
6		3.13	9.428	5.026	4.856
7		25	84.459	4.074	3.935
8		100	322.581	3.489	3.456
9		100	259.740	3.585	3.877
10		12.5	32.895	4.481	4.598

Table 6 Lipinski data for proposed compounds

Comp. no	Mol. formula	H-bond donors	H-bond acceptors	Mol. wt.	CLogP	Lipinski number	Absorption character
6	$C_{15}H_{10}BrNO_3$	0	1	332.15	4.253	4	Good
7	$C_{17}H_{16}N_2O_3$	0	1	296.32	3.557	4	Good
8	$C_{17}H_{18}N_4O_2$	1	1	310.35	3.195	4	Good
9	$C_{19}H_{19}N_3O_6$	1	5	385.37	3.160	4	Good
10	$C_{16}H_{11}Cl_2N_3O_2S$	1	2	380.25	4.806	4	Good

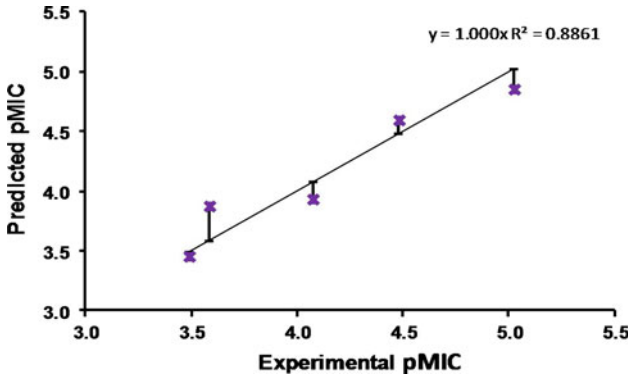


Fig. 4 A *plot* of experimental and predicted pMIC of proposed compounds obtained from selected model

*3-(4-(Dimethylamino) phenyl)-1-(4-nitrophenyl)prop-2-en-1-one (**7**)*

M.p. 210–212 °C; Yield—67 %; IR (ν in cm^{-1}): 3047 (C–H), 2909 (C–H), 1653 (C=O), 1598 (Ar–C=C), 1520 (asym, Ar–NO_2), 1340 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.36–8.28 (m, 4H, Ar–H), 7.75–7.71 (m, 3H, Ar–H and CH=CH), 7.64–7.60 (d, 1H, CH=CH, $J = 11.7$ Hz), 6.77–6.74 (d, 2H, Ar–H, $J = 8.7$ Hz), 3.02 (s, 6H, $N(CH_3)_2$); FAB–MS: *m/z* 297 ($M+H^+$).

*N,N-dimethyl-4-(3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)aniline (**8**)*

M.p. 108–110 °C; Yield—63 %; IR (ν in cm^{-1}): 3389 (NH), 2930 (C–H), 1590 (Ar–C=C), 1508 (asym, Ar–NO_2), 1336 (sym, Ar–NO_2); ^{1}H NMR DMSO (δ in PPM): 8.27–8.20 (m, 2H, Ar–H), 7.79–7.76 (d, 2H, Ar–H,, $J = 9$ Hz), 7.28–7.20 (m, 2H, Ar–H), 6.80–6.68 (m, 2H, Ar–H), 6.22 (bs, 1H, NH), 4.97–4.90 (dd, 1H, CH, $J = 9.0$, 10.8 Hz), 3.48–3.39 (dd, 1H, CH_2, $J = 11.0$, 16.4 Hz), 3.09–3.06 (dd, 1H, CH_2, $J = 8.0$, 9.6 Hz), 2.94 (s, 6H, $N(CH_3)_2$); FAB–MS: *m/z* 311 ($M+H^+$).

*4-(4-Nitrophenyl)-6-(3,4,5-trimethoxyphenyl)-5,6-dihydropyrimidin-2(1H)-one (**9**)*

M.p. >240 °C; Yield—68 %; IR (ν in cm^{-1}): 3172 (NH), 2973 (C–H), 1651 (C=O), 1589 (Ar–C=C), 1506 (asym, Ar–NO_2), 1334 (sym, Ar–NO_2), 1239 (–OCH_3); ^{1}H NMR DMSO (δ in PPM): 8.24–8.21 (m, 3H, Ar–H and NH), 7.84–7.80 (d, 2H, Ar–H, $J = 11.8$ Hz), 6.72 (s, 2H, Ar–H), 5.27–5.13 (m, 2H, CH_2), 4.56 (1H, m, CH), 3.77 (s, 6H, OCH_3), 3.64 (s, 3H, OCH_3); FAB–MS: *m/z* 386 ($M+H^+$).

*6-(2,4-Dichlorophenyl)-4-(4-nitrophenyl)-5,6-dihydropyrimidine-2(1H)-thione (**10**)*

M.p. 208–210 °C; Yield—69 %; IR (ν in cm^{-1}): 3131 (NH), 2932 (C–H), 1597 (Ar–C=C), 1515 (asym, Ar–NO_2), 1331 (sym, Ar–NO_2), 1172 (C=S); ^{1}H NMR DMSO (δ in PPM): 9.28 (s, 1H, NH), 8.28–8.20 (m, 2H, Ar–H), 7.81–7.76 (m, 2H, Ar–H), 7.58–7.54 (d, 1H, Ar–H, $J = 11.7$ Hz), 7.44–7.42 (d, 1H, Ar–H, $J = 6$ Hz), 7.26 (s, 1H, Ar–H), 5.31–5.20 (m, 2H, CH_2), 4.78 (m, 1H, CH); FAB–MS: *m/z* 380 ($M+H^+$).

Conclusion

In conclusion, nitroaryl derivatives of chalcone, pyrazole, isoxazole, and pyrimidine were synthesized and evaluated as anti-tubercular agents. Preliminary structure activity relationship analysis revealed that chalcone analogs are more potent in comparison to substituted pyrazole, isoxazole, pyrimidin-2(1*H*)-one, and pyrimidine-2(1*H*)-thione derivatives. QSAR analysis was performed on set of compounds and key features were identified for the anti-tubercular activity. On the basis of contributing descriptors, new analogs were designed, synthesized, and evaluated. The predicted activity of these compounds is comparable to their experimental pMIC. The newly synthesized chalcones exhibited promising anti-tubercular activities against *M. tuberculosis*. It can be concluded that developed QSAR model could be used for the prediction of newer analogs and further study.

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Conflict of interest The authors report no conflict of interest.

References

Akaike H (1973) Information theory and an extension of the minimum likelihood principle. Akademiai Kiado, Budapest, pp 267–281

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19:716–723

Alland D, Kalkut GE, Moss AR, McAdam RA, Hahn JA, Bosworth W, Drucker E, Bloom BR (1994) Transmission of tuberculosis in New York city—an analysis by DNA fingerprinting and conventional epidemiologic methods. N Engl J Med 330:1710–1716

Bryant D, McCalla D, Leeksma M, Laneuville P (1981) Type I nitroreductases of *Escherichia coli*. Can J Microbiol 27:81–86

Chatterjee S, Hadi A, Price B (2000) Regression analysis by examples, 3rd edn. Wiley, New York

Churkin YD, Panfilova LV, Boreko EI (1982) Synthesis and antiviral activity of unsaturated ketones of thiopene series. Pharm Chem 16:103–105

Corbett EL, Watt CJ, Walker N, Maher BMD, Williams BG, Raviglione MC, Dye C (2003) The growing burden of tuberculosis—global trends and interactions with the HIV epidemic. Arch Intern Med 163:1009–1021

CS ChemOffice (2000) Version 6.0, Cambridge Soft Corporation, Software Publishers Association, 1730 M Street, Suite 700, Washington DC. 20036 (202) 452–1600, USA

Duncan K (2003) Progress in TB drug development and what is still needed. Tuberculosis 83:201–207

Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC (1999) Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. JAMA 282:677–686

Edwards DI (1993) Nitroimidazole drugs-action and resistance mechanisms. I. Mechanisms of action. J Antimicrob Chemother 31:9–20

Friedman J (1990) Multivariate adaptive regression splines. Technical Report No. 102. Laboratory for Computational Statistics. Stanford University, Stanford

Global Alliance for TB Drug Development (2001) Scientific blueprint for tuberculosis drug development. Tuberculosis 81 (Suppl. 1):1–52

Gupta RA, Kaskhedikar SG (2011) Insights into the structural requirement of 6-nitroquinolone-3-carboxylic acids as antimycobacterial agent: chemometric approaches. Med Chem Res. doi: 10.1007/s00044-011-9930-8

Gupta AK, Babu MA, Kaskhedikar SG (2004) VALSTAT: validation program for quantitative structure activity relationship studies. Indian J Pharm Sci 66:396–402

Gupta AK, Gupta RA, Soni LK, Kaskhedikar SG (2008) Exploration of physicochemical properties and molecular modelling studies of 2-sulfonyl-phenyl-3-phenyl-indole analogs as cyclooxygenase-2 inhibitors. Eur J Med Chem 43:1297–1303

Gupta AK, Sabarwal N, Agrawal YP, Prachand S, Jain S (2010) Insights through AM1 calculations into the structural requirement of 3,4,6-substituted-2-quinolone analogs towards FMS kinase inhibitory activity. Eur J Med Chem 45:3472–3479

Hansch C, Leo A (1995) Exploring QSAR, fundamentals and applications in chemistry and biology. American Chemical Society, Washington, DC, pp 90–100

Jacobs RF (1994) Multiple drug resistant tuberculosis. Clin Infect Dis 19:1–10

Jevwon S, Liv CT, Sao LT, Weng JR, Ko HH (2005) Synthetic chalcones as potential anti-inflammatory and cancer chemopreventive agents. Eur J Med Chem 40:103–112

Kaplancikli ZA, Turan-Zitouni G, Ozdemir A, Revial G (2008) New triazole and triazolothiadiazine derivatives as possible antimicrobial agents. Eur J Med Chem 43:155–159

Kier LB (1980) Use of molecular negentropy to encode structure governing biological activity. J Pharm Sci 69:807–810

Kier LB (1986) Shape indexes of orders. One and three from molecular graphs. Quant Struct Act Relat 5:1–7

Kier LB, Hall LH (1983) Estimation of substituent group electronic influence from molecular connectivity delta values. Quant Struct Act Relat 2:163–167

Kier LB, Hall LH (1986) Molecular connectivity in structure–activity analysis. Wiley, Chichester, p 262

Kier LB, Hall LH (1990) An electrotopological state index for atoms in molecules. Pharm Res 7:801–807

Kier LB, Hall LH (1999) Molecular structure description: the electrotopological state. Academic Press, London, p 246

Kubinyi H (1994a) Variable selection in QSAR studies I. An evolutionary algorithm. Quant Struct Act Relat 13:285–294

Kubinyi H (1994b) Variable selection in QSAR Studies II. A highly efficient combination of systematic search and evolution. Quant Struct Act Relat 13:393–401

Lin YM, Flavin MT, Cassidy CS, Mar A, Chen FC (2001) Biflavonoids as novel antituberculosis agents. Bioorg Med Chem Lett 11:2101–2104

Lin YM, Zhou Y, Flavin MT, Zhou LM, Nie W, Chen FC (2002) Chalcones and flavonoids as anti-tuberculosis agents. Bioorg Med Chem 10:2795–2802

Mandloi D, Joshi S, Khadikar PV, Khosla N (2005) QSAR study on the antibacterial activity of some sulfa drugs: building blockers of Mannich bases. Bioorg Med Chem Lett 15:405–411

Nayyar A, Jain R (2005) Recent advances in new structural classes of anti-tuberculosis agents. Curr Med Chem 12:1873–1876

O'Brien RJ, Nunn PP (2001) The need for new drugs against tuberculosis. Am J Respir Crit Care Med 163:1055–1058

Painea SW, Barton P, Bird J, Denton R, Menochet K, Smith A, Tomkinson NP, Chohan KK (2010) A rapid computational filter for predicting the rate of human renal clearance. J Mol Graph Model 29:529–537

Pogliani L (1994) Structure property relationships of amino acids and some dipeptides. Amino Acids 6:141–153

Pogliani L (1996) Modeling with special descriptors derived from a medium-sized set of connectivity indices. J Phys Chem 100: 18065–18077

Prasad YR, Kumar PP, Kumar PR (2008) Synthesis and antimicrobial activity of some new chalcones of 2-acetyl pyridine. Eur J Chem 5:144–148

Shapiro S, Guggenheim B (1998) Inhibition of oral bacteria by phenolic compounda. Part. 1. QSAR analysis using molecular connectivity. Quant Struct Act Relat 17:327–337

Shivakumar PM, Geetha Babu SM, Mukesh D (2005) QSAR studies on chalcones and flavonoids as antitubercular agents using genetic function approximation (GFA) method. Chem Pharm Bull 55:44–49

Smith CV, Sharma V, Sacchettini JC (2004) TB drug discovery: addressing issues of persistence and resistance. Tuberculosis 84:45–55

Todeschini R, Consonni V (2001) DRAGON-Software for the calculation of molecular descriptors, rel. 1.12 for Windows

Weltman AC, Rose DN (1994) Tuberculosis susceptibility patterns, predictors of multidrug resistance, and implications for initial

therapeutic regimens at a New York City hospital. Arch Intern Med 154:2161–2167

WHO. WHO Report (2007) http://www.who.int/tb/publications/global_report/2007/pdf/full.pdf. Accessed Dec 2010

World Health Organization (2003) The WHO Global Tuberculosis Program. http://www.who.int/gtb/. Accessed Sept 2009

Yao XJ, Panaye JP, Doucet JP, Zhang RS, Chen HF, Lice MC, Hu ZD, Fan BT (2004) Comparative study of QSAR/QSPR correlations using support vector machines, radial basis function neural networks, and multiple linear regression. J Chem Inf Comput Sci 44:1257–1266

Zhoua YP, Cai CB, Huanb S, Jiang JH, Wu HL, Shen GL, Yu RQ (2007) QSAR study of angiotensin II antagonists using robust boosting partial least square regression. Anal Chim Acta 593:68–74