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ORIGINAL ARTICLE

Synthesis and antimicrobial activity of N^1-(3-chloro-4-fluorophenyl)-N^4-substituted semicarbazone derivatives

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Abstract A series of 16 N^1-(3-chloro-4-fluorophenyl)-N^4-substituted semicarbazone derivatives were synthesized and subjected to computational pharmacokinetic studies to predict molecular properties. All the title compounds (**4a–p**) followed the Lipinski "Rule of Five". The synthesized compounds were characterized by elemental analyses and spectral data and the compounds (**4a–p**) were evaluated for antimicrobial activities. Among them the compound 2-(4-hydroxybenzylidene)-*N*-(3-chloro-4-fluorolphenyl)hydrazinecarboxamide (**4f**) was found to be the most active compound that showed good antibacterial activity while the compound 2-(4-methoxybenzylidene)-*N*-(3-chloro-4-fluorolphenyl)hydrazinecarboxamide (**4g**) was moderately active against fungal strains. We have noticed that the compounds, (**4f**, **4k** and **4d**) bearing OH and NO_2 groups on the phenyl ring at position 4 exhibited good antibacterial activity while compound (**4g**) bearing OCH_3 on the phenyl ring at position 4 exhibited moderate antifungal activity.

1. Introduction

A wide variety of antibiotics have been developed to combat against bacterial infections. After years of extensive overuse or misuse of antibiotics, bacteria are becoming antibiotic resistant resulting in growing threat to human health around the world. Antibacterial resistance is a cause of increased mortality (Mohamed et al., 2004). Fungal infections like Candidiasis, Cryptococcosis and Aspergillosis are more common in immuno-compromised patients (Spratt, 1994). Life threatening infectious disease caused by multidrug-resistant Gram-positive

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and -negative pathogenic bacteria increased to an alarming level around the world. Owing to this increased microbial resistance, new classes of antimicrobial agents with novel mechanisms are today's need to combat multidrug-resistant infections. Semicarbazones were found to possess different biological activities, such as anticonvulsant (Yogeeswari et al., 2005), antitubercular (Sriram et al., 2004), anticancer (Afrasiabi et al., 2005), antimicrobial (Kasuga et al., 2001), etc. Therefore, in the current work we have focussed on the syntheses of some semicarbazones and their antibacterial and antifungal activities. Earlier we reported the anticonvulsant activity of semicarbazone derivatives (Amir et al., 2010).

2. Molecular properties prediction

Molecular properties, mainly hydrophobicity, molecular size, flexibility and the presence of various pharmacophoric features influence the behavior of molecules in the living organism, including bioavailability. Thus in order to achieve good oral drugs we have subjected a series of semicarbazone derivatives (**4a–p**) for the prediction of lipophilicity, solubility and Lipinski "Rule of Five" and other properties for filtering compounds for antimicrobial screening.

2.1. Lipophilicity

All the compounds were subjected to computational study in order to filter the drugs for antimicrobial screening. For good membrane permeability $\log P$ value should be $\leqslant 5$ (Lipinski et al., 1997). All the title compounds (**4a–p**) have $\log P$ value 3.44–5.467.

2.2. Absorption, polar surface area, and "Rule of Five" properties

Good intestinal absorption, reduced molecular flexibility (measured by the number of rotatable bonds), low polar surface area or total hydrogen bond count (sum of donors and acceptors) are important predictors of good oral bioavailability (Veber et al., 2002; Refsgaard et al., 2005). Membrane permeability and bioavailability are always associated with some basic molecular descriptors such as $\log P$ (partition coefficient), molecular weight (MW), or hydrogen bond acceptors and donors' counts in a molecule. Number of rotatable bonds is important for conformational changes of molecules under study and ultimately for the binding of receptors or channels. It is revealed that for passing oral bioavailability criteria, the number of rotatable bond should be $\leqslant 10$ (Veber et al., 2002). The compounds in this series (**4a–p**) in general possess sufficient number of rotatable bonds (3–5) and therefore, exhibit good conformational flexibility. Molecular polar surface area (TPSA) is a very useful parameter for the prediction of drug transport properties. TPSA is a sum of surfaces of polar atoms (usually oxygen, nitrogen and attached hydrogen) in a molecule. TPSA and volume are inversely proportional to %ABS. TPSA was used to calculate the percentage of absorption (%ABS) according to the equation: $\%ABS = 109 \pm 0.345 \times TPSA$ (Wang et al., 1997). From all these parameters, it can be observed that all the title compounds exhibited a great %ABS ranging from 74% to 99%. The Lipinski "Rule of Five" states that the molecules with good membrane permeability have $\log P \leqslant 5$, molecular weight $\leqslant 500$, number of hydrogen bond acceptors $\leqslant 10$, and number of hydrogen bond donors $\leqslant 5$ (Lipinski et al., 1997). This rule is widely used as a filter for drug-like properties. All the title compounds (**4a–p**) followed the Lipinski "Rule of Five". The pharmacokinetic parameters were calculated online from Molinspiration Chemoinformatics (http://www.molinspiration.com/cgi-bin/properties) and are given in Table 1.

3. Experimental

3.1. Instrumentation

The entire chemical reagents which are used in the study are procured locally. The completion of reaction is monitored by thin layer chromatography (TLC) using chloroform-methanol (9:1) as the solvent system. The products were purified by recrystallisation with absolute ethanol and purity of the compounds was checked by thin layer chromatography (TLC) using silica gel G plates (Merck). The spot was developed in iodine chamber or viewed under UV lamp. Melting points were determined in an open capillary using melting point apparatus and are uncorrected. The proton magnetic resonance (^{1}H NMR) spectra were recorded on a Brucker 300 MHz instrument in DMSOd_6 using tetramethylsilane as an internal standard. The infrared spectra of compounds were recorded in KBr on a Bio-Rad FTIR Spectro-photometer.

3.1.1. Procedure for the synthesis of 3-chloro-4-fluorophenyl urea (2)

3-Chloro-4-fluoro aniline (0.1 mol, 14.65 g) was dissolved in 20 ml of glacial acetic acid and 80 ml of hot water. To this, a solution of sodium cyanate (0.1 mol, 6.5 g) in 80 ml of hot water was added with stirring. It was allowed to stand for 30 min, then cooled in ice bath and filtered with suction, dried and recrystallized from boiling water (Amir et al., 2010). The purity of the compound was checked by TLC using chloroform-methanol (9:1) as the mobile phase. Yield 78%; m.p. 141–143 °C, IR (KBr) cm^{-1}: 3374 (N—H), 1675 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm: 5.92 (s, 2H, NH_2), 7.16–7.23 (m, 2H, aromatic), 7.72–7.74 (d, 1H, aromatic), δ 8.69 (s, 1H, ArH); MS: m/z 189 $M^+ + 1$. Anal. Calcd for $C_7H_6ClFN_2O$: C, 44.58; H, 3.21; N, 14.85. Found: C, 44.56; H, 3.22; N, 14.87.

3.1.2. Procedure for the synthesis of 3-chloro-4-flourophenyl semicarbazide (3)

A mixture of 3-chloro-4-fluorophenyl urea **2** (0.05 mol, 9.45 g) and hydrazine hydrate (0.05 mol, 2.5 ml) in ethanol were refluxed for 48 h with stirring. Two third volume of alcohol was distilled by vacuum distillation and then poured into crushed ice. The resultant precipitate was filtered, washed with water and dried. The solid was recrystallized from 50 ml of 90% ethanol. The purity of the compound was checked by TLC using chloroform-methanol (9:1) as the mobile phase. Yield 66%; m.p. 90–92 °C, IR (KBr) cm^{-1}: 3372 (N—H), 1676 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6) δ ppm: 5.96 (s, 2H, NH_2), 7.20–7.76 (m, 3H, aromatic), 7.89 (s, 1H, Ar-NH), δ 8.88 (s, 1H, CONH); MS: m/z 204 $M^+ + 1$. Anal. Calcd for $C_7H_7ClFN_3O$: C, 41.29; H, 3.47; N, 20.64. Found: C, 41.31; H, 3.49; N, 20.66.

Table 1 Pharmacokinetic parameters important for good oral bioavailability of title compounds (**4a**–**p**).

Compd.	% ABS	Volume (A3)	TPSA (A2)	NROTB	n-ON acceptor	n-OHNH donors	miLog *P*	MW	Lipinski's violations
Rule	–	–	–	–	< 10	< 5	⩽5	< 500	⩽1
4a	90.55	254.504	53.489	3	4	2	4.813	326	0
4b	90.55	254.504	53.489	3	4	2	4.861	326	0
4c	74.74	264.302	99.313	4	7	2	4.094	336	0
4d	74.74	264.302	99.313	4	7	2	4.142	336	0
4e	83.57	248.986	73.717	3	5	3	4.123	307	0
4f	83.57	248.986	73.717	3	5	3	3.704	307	0
4g	87.36	266.514	62.723	4	5	2	4.24	321	0
4h	84.17	292.059	71.957	5	6	2	3.829	351	0
4i	89.43	286.874	56.727	4	5	2	4.285	334	0
4j	90.55	268.04	53.489	3	4	2	5.467	360	1
4k	83.57	265.547	73.717	3	5	3	3.617	321	0
4l	87.36	283.075	62.723	4	5	2	4.153	335	0
4m	99.55	271.065	53.489	3	4	2	4.774	340	0
4n	74.74	280.863	99.313	4	7	2	4.055	350	0
4o	99.55	274.09	53.489	3	4	2	4.545	319	0
4p	86.01	222.536	66.629	3	5	2	3.44	281	0

%ABS, percentage of absorption; TPSA, topological polar surface area; NROTB, number of rotatable bonds; MW, molecular weight; miLog *P*, logarithm of compound partition coefficient between n-octanol and water; *n*-OHNH, number of hydrogen bond donors; *n*-ON, number of hydrogen bond acceptors.

*3.1.3. General procedure for the synthesis of N^1-(3-chloro-4-fluorophenyl)-N^4-substituted semicarbazones (**4a–p**)*

To a solution of 3-chloro-4-fluorophenyl semicarbazide **2** (0.005 mol, 1.02 g) in 1 ml conc. HCl and 25 ml water was added sodium acetate solution (0.005 mol, 0.41 g in 2 ml water). About 25 ml ethanol was added to clear the turbidity. This solution mixture was added to an equimolar quantity of the appropriate aldehydes or ketones in alcohol. The solution was stirred for 15 min immediately precipitation occurred, (if immediate precipitation did not occurred, reaction mixture was allowed to stand for 1–2 h.) and the solids were filtered, dried and recrystallized from hot ethanol. The completion of reactions was monitored by TLC using chloroform-methanol (9:1) as the mobile phase.

*3.1.3.1. 2-(2-Chlorobenzylidene)-N-(3-chloro-4-fluorophenyl) hydrazinecarboxamide (**4a**).* Yield 72%; m.p. 178–180 °C, IR (KBr) cm^{-1}: 3375 (N—H), 1677 (C═O), 1532 (C═N); ^1H NMR (DMSO-d_6) δ ppm: 7.22–7.94 (m, 7H, aromatic), 7.94 (s, 1H, N═CH), 9.19 (s, 1H, Ar–NH), 11.11 (s, 1H, CONH); MS: *m*/*z*, M^+ 325, $M^+ + 1$ 326, $M^+ + 2$ 327. Anal. Calcd for $C_{14}H_{10}Cl_2FN_3O$: C, 51.56; H, 3.09; N, 12.88. Found: C, 51.57; H, 3.10; N, 12.87.

*3.1.3.2. 2-(4-Chlorobenzylidene)-N-(3-chloro-4-fluorophenyl) hydrazinecarboxamide (**4b**).* Yield 72%; m.p. 190–192 °C, IR (KBr) cm^{-1}: 3400 (N—H), 1694 (C═O), 1536 (C═N); ^1H NMR (DMSO-d_6) δ ppm: 7.02–7.84 (m, 7H, aromatic), 7.96 (s, 1H, N═CH), 9.10 (s, 1H, Ar–NH), 11.13 (s, 1H, CONH); MS: *m*/*z*, M^+ 325, $M^+ + 1$ 326, $M^+ + 2$ 327. Anal. Calcd for $C_{14}H_{10}Cl_2FN_3O$: C,51.56; H, 3.09; N, 12.88. Found: C, 51.57; H, 3.10; N, 12.87.

*3.1.3.3. 2-(2-Nitrobenzylidene)-N-(3-chloro-4-fluorophenyl) hydrazinecarboxamide (**4c**).* Yield 72%; m.p. 218–220 °C, IR (KBr) cm^{-1}: 3403 (N—H), 1690 (C═O), 1526 (C═N); ^1H NMR (DMSO-d_6) δ ppm: 7.22–7.87 (m, 7H, aromatic), 7.99 (s, 1H, N═CH), 9.20 (s, 1H, Ar-NH), 11.23 (s, 1H, CONH); MS: *m*/*z*, M^+ 336, $M^+ + 1$ 337, $M^+ + 2$ 338. Anal. Calcd for $C_{14}H_{10}ClFN_4O_3$: C, 49.94; H, 2.99; N, 16.64. Found: C, 49.94; H, 3.00; N, 16.66.

*3.1.3.4. 2-(4-Nitrobenzylidene)-N-(3-chloro-4-fluorophenyl) hydrazinecarboxamide (**4d**).* Yield 68%; m.p. 198–200 °C, IR (KBr) cm^{-1}: 3403 (N—H), 1690 (C═O), 1526 (C═N); ^1H NMR (DMSO-d_6) δ ppm: 7.02–7.67 (m, 7H, aromatic), 7.89 (s, 1H, N═CH), 9.19 (s, 1H, Ar–NH), 11.43 (s, 1H, CONH); MS: *m*/*z*, M^+ 336, $M^+ + 1$ 337, $M^+ + 2$ 338. Anal. Calcd for $C_{14}H_{10}ClFN_4O_3$: C, 49.94; H, 2.99; N, 16.64. Found: C, 49.94; H, 3.00; N, 16.66.

*3.1.3.5. 2-(2-Hydroxybenzylidene)-N-(3-chloro-4-fluorophenyl) hydrazinecarboxamide (**4e**).* Yield 58%; m.p. 173–175 °C, IR (KBr) cm^{-1}: 3375 (N—H), 1676 (C═O), 1536 (C═N); (DMSO-d_6) δ ppm: 7.22–8.38 (m, 7H, aromatic), 8.48 (s, 1H, N═CH), 9.15 (s, 1H, Ar–NH), 10.60 (s, 1H, Ar-OH), 11.18 (s, 1H, CONH); MS: *m*/*z*, M^+ 307, $M^+ + 1$, 308, $M^+ + 2$, 309; Anal. Calcd for $C_{14}H_{11}ClFN_3O_2$: C, 54.65; H, 3.60; N, 13.66. Found: C, 54.67; H, 3.59; N, 13.65.

*3.1.3.6. 2-(4-Hydroxybenzylidene)-N-(3-chloro-4-fluorophenyl) hydrazinecarboxamide (**4f**).* Yield 68%; m.p. 202–204 °C, IR (KBr) cm^{-1}: 3370 (N—H), 1678 (C═O), 1536 (C═N); (DMSO-d_6) δ ppm: 6.79–7.95 (m, 7H, aromatic), 7.97 (s, 1H, N═CH), 9.02 (s, 1H, Ar–NH), 9.85 (s, 1H, CONH), 10.67 (s, 1H, Ar-OH); MS: *m*/*z*, M^+ 307, $M^+ + 1$, 308, $M^+ + 2$, 309; Anal. Calcd for $C_{14}H_{11}ClFN_3O_2$: C, 54.65; H, 3.60; N, 13.66. Found: C, 54.66; H, 3.60; N, 13.67.

*3.1.3.7. 2-(4-Methoxybenzylidene)-N-(3-chloro-4-fluorophenyl) hydrazinecarboxamide (**4g**).* Yield 70%; m.p. 148–150 °C, IR (KBr) cm^{-1}: 3360 (N—H), 1688 (C═O), 1546 (C═N);

(DMSO-d_6) δ ppm: 3.75 (s, 3H. OCH_3), 6.79–7.90 (m, 7H, aromatic), 7.98 (s, 1H, N═CH), 9.08 (s, 1H, Ar–NH), 11.05 (s, 1H, CONH); MS: m/z, M^+ 321, $M^+ + 1$, 322, $M^+ + 2$, 323; Anal. Calcd for $C_{15}H_{13}ClFN_3O_2$: C, 56.00; H, 4.07; N, 13.06. Found: C, 56.03; H, 4.10; N, 13.07.

*3.1.3.8. 2-(3,4-Dimethoxybenzylidene)-N-(3-chloro-4-fluorophenyl)hydrazinecarboxamide (**4h**).* Yield 70%; m.p. 192–194 °C, IR (KBr) cm^{-1}: 3370 (N─H), 1678 (C═O), 1576 (C═N); (DMSO-d_6) δ ppm: 3.80 (s, 6H. $(OCH_3)_2$), 6.80–7.91 (m, 6H, aromatic), 7.90 (s, 1H, N═CH), 9.09 (s, 1H, Ar-NH), 11.00 (s, 1H, CONH); MS: m/z M^+ 351, $M^+ + 1$, 352, $M^+ + 2$, 353; Anal. Calcd for $C_{16}H_{15}ClFN_3O_3$: C, 54.63; H, 4.30; N, 11.95. Found: C, 54.63; H, 4.32; N, 11.96.

*3.1.3.9. 2-(4,N,N-Dimethylaminobenzylidene)-N-(3-chloro-4-fluorophenyl)hydrazinecarboxamide (**4i**).* Yield 74%; m.p. 182–184 °C, IR (KBr) cm^{-1}: 3366 (N─H), 1678 (C═O), 1538 (C═N); (DMSO-d_6) δ ppm: 2.96 (s, 6H, $N(CH_3)_2$), 6.70–7.82 (m, 7H, aromatic), 7.97(s, 1H, N═CH), 9.01 (s, 1H, Ar–NH), 10.59 (s, 1H, CONH); MS: m/z, M^+ 334, $M^+ + 1$, 335, $M^+ + 2$, 336; Anal. Calcd for $C_{16}H_{16}ClFN_4O$: C, 57.40; H, 4.82; N, 16.74. Found: C, 57.42; H, 4.81; N, 16.72.

*3.1.3.10. 2-(2,4-Dichlorobenzylidene)-N-(3-chloro-4-fluorophenyl)hydrazinecarboxamide (**4j**).* Yield 66%; m.p. 144–146 °C, IR (KBr) cm^{-1}: 3376 (N─H), 1674 (C═O), 1548 (C═N); (DMSO-d_6) δ ppm: 2.98 (s, 6H, $N(CH_3)_2$), 6.77–7.82 (m, 6H, aromatic), 7.99 (s, 1H, N═CH), 9.06 (s, 1H, Ar–NH), 10.57 (s, 1H, CONH); MS: m/z, M^+ 360, $M^+ + 1$, 361, $M^+ + 2$, 362; Anal. Calcd for $C_{16}H_{16}ClFN_4O$: C, 46.43; H, 2.52; N, 11.65. Found: C, 46.64; H, 2.53; N, 11.66.

*3.1.3.11. 2-[1-(4-Hydroxyphenyl)ethylidene]-N-(3-chloro-4-fluorophenyl)hydrazinecarboxamide (**4k**).* Yield 66%; m.p. 180–182 °C, IR (KBr) cm^{-1}: 3373 (N─H), 1686 (C═O), 1528 (N═C); (DMSO-d_6) δ ppm: 2.23 (s, 3H, CH_3), 6.70–7.82 (m, 7H, aromatic), 9.09 (s, 1H, Ar–NH), 10.59 (s, 1H, CONH), 12.06 (s,1H, OH); MS: m/z, (M^+) 321, $M^+ + 1$, 322, $M^+ + 2$, 323; Anal. Calcd for $C_{15}H_{13}ClFN_3O_2$: C, 56.00; H, 4.07; N, 13.06. Found: C, 56.02; H, 4.08; N, 13.08.

*3.1.3.12. 2-[1-(4-Methoxyphenyl)ethylidene]-N-(3-chloro-4-fluorophenyl)hydrazinecarboxamide (**4l**).* Yield 72%; m.p. 140–142 °C, IR (KBr) cm^{-1}: 3369 (N─H), 1669 (C═O), 1541 (C═N); (DMSO-d_6) δ ppm: 2.23 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 6.94–7.96 (m, 7H, aromatic), 9.05 (s, 1H, Ar–NH), 9.83 (s, 1H, CONH); MS: m/z, M^+ 321, $M^+ + 1$, 322, $M^+ + 2$, 323; Anal. Calcd for $C_{16}H_{15}ClFN_3O_2$: C, 57.23; H, 4.50; N, 12.51. Found: C, 57.20; H, 4.48; N, 12.53.

*3.1.3.13. 2-[1-(4-Chlorophenyl)ethylidene]-N-(3-chloro-4-fluorophenyl)hydrazinecarboxamide (**4m**).* Yield 70%; m.p. 270–272 °C, IR (KBr) cm^{-1}: 3360 (N─H), 1679 (C═O), 1549 (C═N); (DMSO-d_6) δ ppm: 2.28 (s, 3H, CH_3), 6.99–7.99 (m, 7H, aromatic), 9.00 (s, 1H, Ar–NH), 9.99 (s, 1H, CONH); MS: m/z, M^+ 335, $M^+ + 1$, 336, $M^+ + 2$, 337; Anal. Calcd for $C_{15}H_{12}Cl_2FN_3O$: C, 52.56; H, 3.56; N, 12.35. Found: C, 52.58; H, 3.58; N, 12.36.

*3.1.3.14. 2-[1-(4-Nitrophenyl)ethylidene]-N-(3-chloro-4-fluorophenyl)hydrazinecarboxamide (**4n**).* Yield 70%; m.p. 186–188 °C, IR (KBr) cm^{-1}: 3369 (N─H), 1675 (C═O), 1579 (C═N); (DMSO-d_6) δ ppm: 2.18 (s, 3H, CH_3), 6.89–7.79 (m, 7H, aromatic), 9.05 (s, 1H, Ar–NH), 10.29 (s, 1H, CONH); MS: m/z, M^+ 350, $M^+ + 1$, 351, $M^+ + 2$, 352; Anal. Calcd for $C_{15}H_{12}ClFN_4O_3$: C, 51.37; H, 3.45; N, 15.97. Found: C, 51.38; H, 3.46; N, 15.99.

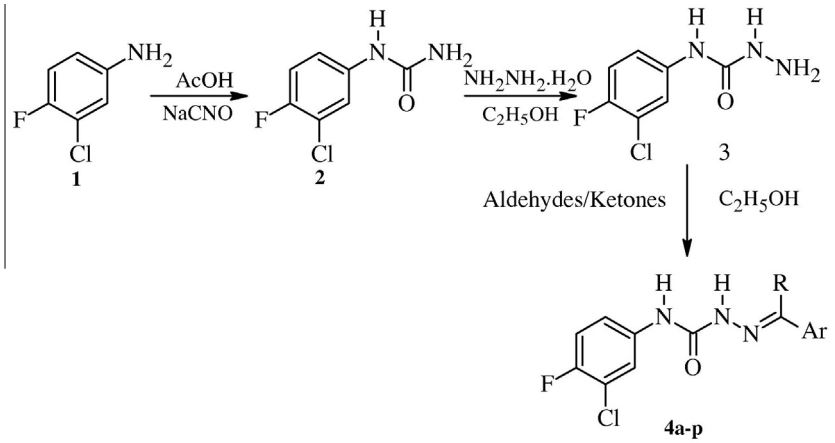
*3.1.3.15. 2-[1-(4-Methylphenyl)ethylidene]-N-(3-chloro-4-fluorophenyl)hydrazinecarboxamide (**4o**).* Yield 72%; m.p. 176–178 °C, IR (KBr) cm^{-1}: 3367 (N─H), 1680 (C═O), 1540 (C═N); (DMSO-d_6) δ ppm: 2.02 (s, 3H, CH_3), 2.27 (s, 3H, Ar–CH_3), 6.51–7.93 (m, 7H, aromatic), 9.09 (s, 1H, Ar–NH), 9.88 (s, 1H, CONH); MS: m/z, (M^+) 319, $M^+ + 1$, 320, $M^+ + 2$, 321; Anal. Calcd for $C_{16}H_{15}ClFN_3O$: C, 60.10; H, 4.73; N, 13.14. Found: C, 60.13; H, 4.75; N, 13.15.

*3.1.3.16. 2-(Furan-2-ylmethylidene)-N-(3-chloro-4-fluorophenyl)hydrazinecarboxamide (**4p**).* Yield 68%; m.p. 158–160 °C, IR (KBr) cm^{-1}: 3377 (N─H), 1685 (C═O), 1545 (C═N); (DMSO-d_6) δ ppm: 6.51–7.73 (m, 6H, aromatic), 7.99 (s, 1H, N═CH), 8.09 (s, 1H, Ar–NH), 9.18 (s, 1H, CONH); MS: m/z, (M^+) 281, $M^+ + 1$, 282, $M^+ + 2$, 283; Anal. Calcd for $C_{12}H_9ClFN_3O_2$: C, 51.17; H, 3.22; N, 14.92. Found: C, 51.19; H, 3.23; N, 14.93.

4. Results and discussion

4.1. Chemistry

The title compounds (**4a–p**) were described in this study and the reaction sequence for the synthesis is summarised in Scheme 1. In the initial step 3-chloro-4-fluorophenyl urea (**2**) was synthesized from sodium cyanate and 3-chloro-4-fluoroaniline (**1**) in glacial acetic acid which was then refluxed with hydrazine hydrate in ethanol to obtain 3-chloro-4-fluorophenyl semicarbazides (**3**) followed by condensation with appropriate aldehydes or ketones in the presence of ethanol and sodium acetate furnished the titled compounds (**4a–p**). The yields of the titled compounds were ranging from 58% to 74% after recrystallization with absolute ethanol. The purity of the compounds was checked by TLC using eluants chloroform-methanol (9:1) and elemental analyses. Both the analytical and spectral data (IR, ^1H NMR) of all the synthesized compounds were in full agreement with the proposed structures. In general, infra red spectra (IR) revealed N─H, C═O, and C═N at 3366–3375, 1679–1686, and 1528–1541 cm^{-1}, respectively. In the Nuclear Magnetic resonance spectra (^1H NMR) the signals of the respective protons of the prepared titled were verified on the basis of their chemical shift, multiplicities and coupling constants. The spectra showed a singlet at δ 2.02–2.96 ppm corresponding to CH_3 group; singlet at δ 3.79–3.80 ppm corresponding to OCH_3 group; multiplet at δ 6.51–8.38 ppm corresponding to aromatic protons; singlet at δ 7.89–10.42 ppm corresponding to Ar–NH; singlet at δ 7.96–8.76 ppm corresponding to N═CH; singlet at δ 8.88–11.52 ppm corresponding to CONH; singlet at δ 10.67 ppm corresponding to Ar-OH. The elemental analysis results were within $\pm 0.4\%$ of the theoretical values.



R = H, CH_3; Ar = 2-Cl-C_6H_4, 4-Cl-C_6H_4, 2-NO_2-C_6H_4, 4-NO_2-C_6H_4,2-OH-C_6H_4, 4-OH-C_6H_4, 4-OCH_3-C_6H_4, 3,4-$(OCH_3)_2$-C_6H_3, 4-$N(CH_3)_2$-C_6H_4, 2,4-$(Cl)_2$-C_6H_3, 4-CH_3-C_6H_3, 2-furyl

Scheme 1 Protocol for synthesis.

4.2. Antibacterial activity

The investigation of antibacterial screening data revealed that all the tested compounds showed good to low bacterial inhibition. The compound **4f** showed good antibacterial inhibition against *Escherichia coli* and *Pseudomonas aeruginosa* at 4 μg/ml equivalents to standard Ciprofloxacin. The compounds **4d** and **4k** showed moderate activity between 16 and 32 μg/ml, rest of the compounds showed low antibacterial activity.

4.3. Antifungal activity

Antifungal screening data revealed that most of the compounds showed moderate to low activity. The most active compound of the series was **4g** which showed fungal inhibition against *Aspergillus niger* and *Candida albicans* at MIC of 4 and 8 μg/ml, respectively. The compounds **4h**, **4i**, **4l** and **4o** showed moderate activities between 8 and 16 μg/ml, while rest of the compounds showed low antifungal activity.

5. Conclusion

A series (**4a–p**) of semicarbazone derivatives were subjected for the prediction of molecular properties before antimicrobial screening. All the 16 compounds followed the Lipinski ''Rule of Five''. The syntheses of semicarbazone derivatives were governed by treating aniline with sodium cyanate to obtain phenyl urea (**2**) which was then refluxed with hydrazine hydrate in ethanol to obtain 3-chloro-4-fluorophenyl semicarbazides (**3**) followed by condensation with appropriate aldehydes or ketones in the presence of ethanol and sodium acetate furnished the titled compounds (**4a–p**). All the titled compounds were screened for antibacterial and antifungal activity as per the standard protocol. On the basis of the results obtained from antimicrobial screening it was found that compound 2-(4-hydroxybenzylidene)-*N*-(3-chloro-4-fluorolphenyl)hydrazinecarboxamide (**4f**) was the most active compound that showed good antibacterial activity while the compound 2-(4-methoxybenzylidene)-*N*-(3-chloro-4-fluorolphenyl)hydrazinecarboxamide (**4g**) was moderately active on fungal strains. Examining closely on substitutions, it may be concluded that the electron releasing group such as –OH and electronegative group such as $–NO_2$ on the phenyl ring at position 4 showed good antibacterial activity while the electron releasing group such as $–OCH_3$ on phenyl ring at position 4 showed moderate antifungal activity. Further it may be concluded that the activity was more pronounced if imine H was present on the semicarbazones.

6. Biological methods

6.1. Antibacterial screening

The synthesized title compounds (**4a–p**) were screened for antibacterial activity against *Staphylococcus aureus* (NCIM 2079), *B. subtilis* (NCIM 2439), *E. coli* (NCIM 5051) and *P. aeruginosa* (ATCC 10145) bacterial strains by disc-diffusion method (Cruickshank et al., 1975; Collins, 1976). All the bacterial strains were procured from National Chemical Laboratory, Pune and American Type Culture Collection (ATCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India. Ciprofloxacin was used as a standard drug. Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amount of the test compound and controls was inoculated with approximately 5×10^5 c.f.u. of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). The minimum inhibitory concentrations are given in Table 2.

6.2. Antifungal screening

The synthesized title compounds (**4a–p**) were screened for antifungal activity against *A. niger* (NCIM 1196) and *C. albicans* (NCIM 3471) in DMSO by agar diffusion method (Khan, 1997; Varma, 1998). All the fungal strains were procured from National Chemical Laboratory, Pune, India. The fungal activity of each compound was compared with fluconazole as

Table 2 Antimicrobial screening of the title compounds.

Compound	Minimum inhibitory concentration (μg/ml)					
	Antibacterial				Antifugal	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*A. niger*	*C. albicans*
4a	64	128	64	256	128	128
4b	128	128	128	256	256	128
4c	32	64	32	64	128	64
4d	8	16	8	32	64	32
4e	32	32	32	64	128	256
4f	8	16	4	4	128	256
4g	128	128	64	128	4	8
4h	128	128	64	256	8	16
4i	256	256	256	512	8	8
4j	32	16	16	32	256	256
4k	8	16	8	16	128	128
4l	32	32	32	64	8	16
4m	64	128	128	256	128	128
4n	16	16	16	32	128	64
4o	256	256	128	256	16	16
4p	256	256	256	256	256	256
Ciprofloxacin	4	4	4	4	–	–
Fluconazole	–	–	–	–	2	1

standard drug. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately $1.6–6 \times 10^4$ c.f.u./ml. The cultures were incubated for 48 h at 35 °C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of the fungus was regarded as minimum inhibitory concentration (MIC). The minimum inhibitory concentrations are given in Table 2.

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