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ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · MARCH 2015

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2015.03.021

European Journal of Medicinal Chemistry 95 (2015) 514–525

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Novel imidazo[2,1-*b*]-1,3,4-thiadiazoles as promising antifungal agents against clinical isolate of *Cryptococcus neoformans*

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ARTICLE INFO

Article history:
Received 3 October 2014
Received in revised form
9 March 2015
Accepted 11 March 2015
Available online 12 March 2015

Keywords:
Imdazo [2,1-*b*]-1,3,4-thiadiazole
Chalcones
Schiff bases
Antifungal activity
Cryptococcosis
Cryptococcus neoformans

ABSTRACT

We herein report the synthesis and *in vitro* antimicrobial evaluation of twenty five novel hybrid derivatives of imidazo [2,1-*b*]-1,3,4-thiadiazole containing chalcones (**5a**–**o**) and Schiff bases (**6a**–**j**) against three fungal strains (*Candida albicans*, *Cryptococcus neoformans* and *Aspergillus niger*). Most of the tested compounds displayed substantial anti-fungal activity with MICs ranging between 1.56 and 100 μg/mL. Compounds **5a**, **5b** and **5n** exhibited promising activity against *C. neoformans* at a MIC 1.56 μg/mL. In addition, compound **5n** also demonstrated significant antifungal activity against the clinical isolates of *C. neoformans* at MIC 3.125 μg/mL. However, moderate activity was observed for these compounds against four bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*) and *Mycobacterium tuberculosis* ($H_{37}Rv$).

1. Introduction

In the recent years, mortality and morbidity rate of opportunistic fungal infections is exponentially increasing and the number of fatal incidence due to fungi is becoming comparable with that of tuberculosis and malaria [1]. This could be attributed to increase in the number of patients with organ and stem cell transplantation, HIV/AIDS patients and other immune compromised patients [2]. Furthermore, the primary organisms responsible for invasive fungal infections (*e.g. Candida*, *Cryptococcus* and *Aspergillus* species) have developed drastic resistance. Patients with significant immunosuppression frequently develop *Cryptococcosis*, which is caused by the encapsulated yeast *Cryptococcus neoformans* and is responsible for serious clinical illnesses like lung infections, fungal meningitis and encephalitis [3]. It spreads by gulping aerosolized spores which may enter the pulmonary or the central nervous system [4]. Moreover, the virulence of *C. neoformans* depends upon the strain resistance and the immune level of the host, which could be latent and may lead to a permanent neurological injury [5–7]. A matter of grave concern in the treatment of fungal infections is the availability of limited number of efficacious antifungal drugs (*e.g.* amphotericin B, 5-fluorocytosine, fluconazole and voriconazole), which suffer from severe drawbacks such as; narrow therapeutic spectrum, drug resistance, high toxicity and low bioavailability [8,9]. Although the use of a new generation of triazoles, the available polyenes in lipid formulations, the use of echinocandins or the combination therapy have been introduced as alternatives in the last ten years, but fungal infections still remains difficult to eradicate [10]. This necessitates urgent need to discover and develop novel chemotype antifungal molecules.

The fused ring of imidazole with a 1,3,4-thiadiazole motif is a very important heterocyclic system containing a bridgehead nitrogen atom known as imidazo[2,1-*b*]-1,3,4-thiadiazoles. Imidazo [2,1-*b*]- 1,3,4 thiadiazoles are known to exhibit a diverse array of biological activities such as antibacterial [11–13], anti-fungal (**I**) [14], anti-tubercular [15], anticonvulsant (**II**) [16,17], anti-hyperlipidemic [18], anti-inflammatory (**III**), analgesic, antipyretic [19,20], anti-cancer (**IV**), anthelmintic and anti ambeic agents [21]. Yet the cellular biology and the interactions of these compounds with different receptors and enzymes have not been widely studied

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http://dx.doi.org/10.1016/j.ejmech.2015.03.021
0223-5234/

[22]. Despite numerous attempts to develop new structural prototype in the search for more effective antimicrobials, the imidazo [2,1-*b*]-1,3,4-thiadiazole still remain as one of the most versatile scaffold, which could be further exploited to develop hybrids as promising antimicrobial agents.

On the other hand, the concept of hybrid drugs has gained more attention wherein two or more bioactive pharmacophores are linked covalently to have synergistic effect [23]. It is anticipated that such approach may solve the problem of drug resistance by displaying dual drug action [24]. Using this approach, several research groups have recently reported hybrid molecules by coupling medicinally privileged motif chalcone with biologically important pharmacophores. For instance an integration of coumarins with chalcones (**V**), led to hybrid compounds which displayed potent antitumor activity [25]. Likewise integration of Isatins with chalcones (**VI**) exhibited more efficacious activity than the commonly used chemotherapeutic drug cisplatin against the breast cancer cell lines [26]. Similarly, fusion of bromopyrrole alkaloid with chalcones (**VII**) demonstrated potent cytotoxicity and revealed that the integration of 4,5-dibromopyrrole moiety into chalcones lead to significant improvement of cytotoxic profile of 4,5-dibromopyrrole [27]. Correspondingly, chalcones bearing 2,4-thiazolidinedione and benzoic acid moieties (**VIII**) presented potential anti-bacterial activity against gram positive bacteria, particularly against multidrug-resistant strains of clinical isolates [28]. Moreover, integration of 5-nitroisoquinolines (**IX**) and Abacavir prodrugs (**X**) with Schiff bases enhanced the anti-malarial and anti-HIV scope of these compounds [29,30]. Equally, integrating isoniazid a well-known anti-TB drug with aryl hydrzone led to a hybrid (**XI**) which exhibited potent anti-tubercular activity [31]. Likewise, nitrofurantion hybrid (**XII**) with anti-infective activity was discovered by hybridizing 5-nitrofuran carboxyaldehyde motif with hydrazide feature [32] as illustrated in Fig. 1. Therefore, in view of the above facts and in continuation of our search on biologically active hybrid molecules, herein we report the synthesis and spectral studies of novel chalcones (**5a**–**o**) and Schiff base (**6a**–**j**) hybrids of imidazo[2,1-*b*]-1,3,4-thiadiazoles with their subsequent *in vitro* biological evaluation for antibacterial, antifungal and antimycobacterial activity.

2. Chemistry

The synthesis of a series of novel chalcones (**5a**–**o**) and Schiff base (**6a**–**j**) hybrids of imidazo[2,1-*b*]-1,3,4-thiadiazole was achieved through convenient and efficient synthetic route as outlined in Scheme 1. Synthesis of the desired 2-substitutedphenyl-6-(4-bromophenyl)imidazo(2,1-*b*)1,3,4-thiadiazole (**3a**–**f**) was carried out by the condensation of 2-amino-5-substituted phenyl-1,3,4-thiadiazole (**2a**–**f**) with α-bromo ketone in DMF. Vilsmeier–Haack reaction of compounds (**3a**–**f**) with phosphurs oxychloride and DMF yielded the corresponding derivatives 2-substituted imidazo[2,1-*b*]- 1,3,4thiadiazole-5-carbaldehydes (**4a**–**f**) in good yields (85–90%). The aldehyde functional group at the 5th position of the imidazo[2,1-*b*]1,3,4-thiadiazole nucleus was utilized to perform Claisen–Schmidt reaction with different aryl/heteroaryl ketones in ethanolic NaOH (10%) to afford corresponding chalcone derivatives (**5a**–**o**). Compounds (**4a**–**f**) were further reacted with aliphatic cyclic amine using conventional method by refluxing in ethanol with catalytic amount of glacial acetic acid for 24 h. This resulted in lower yields of Schiff bases (**6a**–**j**) (10–20%). Thus, in order to improve the yield reactions were carried out under controlled microwave irradiation (*CEM* Discover, Explorer-*12 Hybrid*, *Microwave conditions*: 25 min at 150 psi), which afforded the corresponding Schiff base (**6a**–**j**) in good yields (44–83%) [11,27].

3. Results and discussion

Structures of compound (**4a**–**f**) and their corresponding final hybrid derivatives (**5a**–**o** and **6a**–**j**) were characterized based on their physicochemical and spectral (IR, ^{1}H NMR, ^{13}C NMR and MS) analysis. The analytical data of all the newly synthesized compounds along with their anticipated structures are summarized in Supporting information. The IR spectrum of compounds (**4a**–**e**) exhibited prominent and informative band, which appeared around 1757–1676 cm^{-1} indicating the presence of C=O (aldehydic carbonyl) group with confirming the formylation of compounds (**3a**–**e**) by Vilsmeier–Haack reaction. This was further substantiated from ^{1}H NMR spectra of compounds (**4a**–**f**), which exhibited a very distinct singlet peak resonating at δ 10.14–10.09 ppm indicating the presence of aldehydic (CHO) proton, thus confirming the formation of imidazo[2,1-*b*]-1,3,4thiadiazoles-5-carbaldehydes. The formation of title chalcone derivatives (**5a**–**o**) is evident from their IR spectra, wherein the appearance of some prominent characteristic bands around 1674–1642 cm^{-1} due to C=O stretch (chalcone), 1590–1406 cm^{-1} for C=C stretch (α–β unsaturated carbons of chalcone) and 810.2–679 cm^{-1} for C–Br Str. The ^{1}H NMR spectra of compounds (**5a**–**o**) revealed two distinctive doublets at *δ* 8.38–8.17 ppm and *δ* 8.09–8.03 ppm with coupling constant (*J*) of 14–16 Hz, indicating the presence of *α* and *β* unsaturated protons of chalcones, while the various aromatic protons appeared around δ 7.90–7.05 ppm.

The formation of imines (**6a**–**j**) was confirmed by IR spectra, where the disappearance of strong band around 1757–1676 cm^{-1} of C=O group and appearance of characteristic imine (HC=N Str) band between 1671 and 1561 cm^{-1}. Further, the ^{1}H NMR spectra of Schiff base compounds (**6a**–**j**) displayed a prominent singlet signal resonating around δ 8.64–7.97 ppm, which was attributed to imine proton (CH=N), while the aromatic/heteroaromatic protons appeared as doublets/multiplet signals between δ 8.14–6.96 ppm. In ^{13}C NMR spectrum, it was observed that the most characteristic carbon signals (CH_3, OCH_3, –CH=CH– and C=O) appeared at around δ 21.6, 55.6, 150–138.0 and 190.1–181.8 ppm respectively, while the signals observed at around δ 121.5–114.4, 138.5–132.0, 145.9–138.3, 149.9–145.7 ppm were assigned to C-5, C-7a, C-2, C-6. The aromatic carbon peaks appeared around δ 135.0–120.0 ppm, whereas the aliphatic cyclopropyl and/or cyclohexyl carbons resonated between δ 70.4–8.1 ppm. In addition, the formation of title compounds was also confirmed by recording their respective mass spectra, which were in agreement with their expected molecular weights.

The Chalcone (**5a**–**o**) and Schiff base (**6a**–**j**) derivatives of imdazo[2,1-*b*]-1,3,4-thiadiazole scaffold were evaluated for their *in vitro* antifungal activity against *Candida albicans* ATCC90028, *C. neoformans* ATCC6603 and *Aspergillus niger* ATCC16404, where Amphotericin B was used as reference drug. The results (MIC values) of *in vitro* antifungal screening of the test compounds are summarized in Table 1. However, a systematic analysis of the data as depicted in Table 1 revealed that compounds **5a**, **5b** and **5n** exhibited comparable antifungal activity (MICs = 1.56 μg/mL), against *C. neoformans* as the standard drug Amphotericin B (MIC = 1–2 μg/mL).

Compounds **5a**, **5b** and **5n** exhibited most promising activity against *C. neoformans* at a MIC 1.56 μg/mL while compounds **5c**, **5k** and **5m** were moderately active at a MIC 3.125 μg/mL. Compound **6d** (MIC = 12.5 μg/mL) showed moderate antifungal activity against *C. albicans*. Further, all the synthesized compounds were tested against two well characterized clinical isolates of fungal strains *C. albicans* and *C. neoformans*. The antifungal activity on the clinical isolates was carried out at the Department of Microbiology, Inkosi Albert Luthuli Hospital, Durban, South Africa. Among the tested

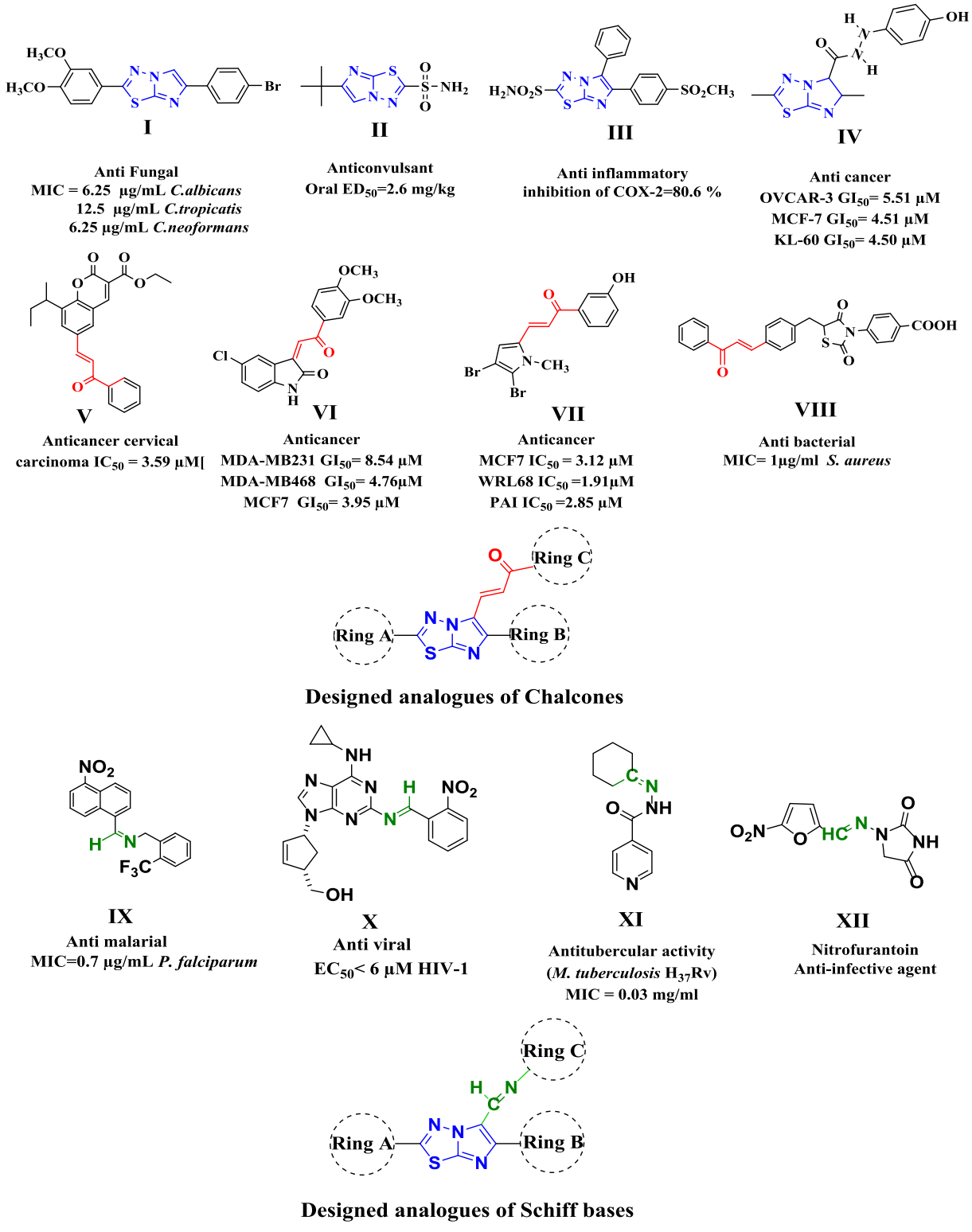
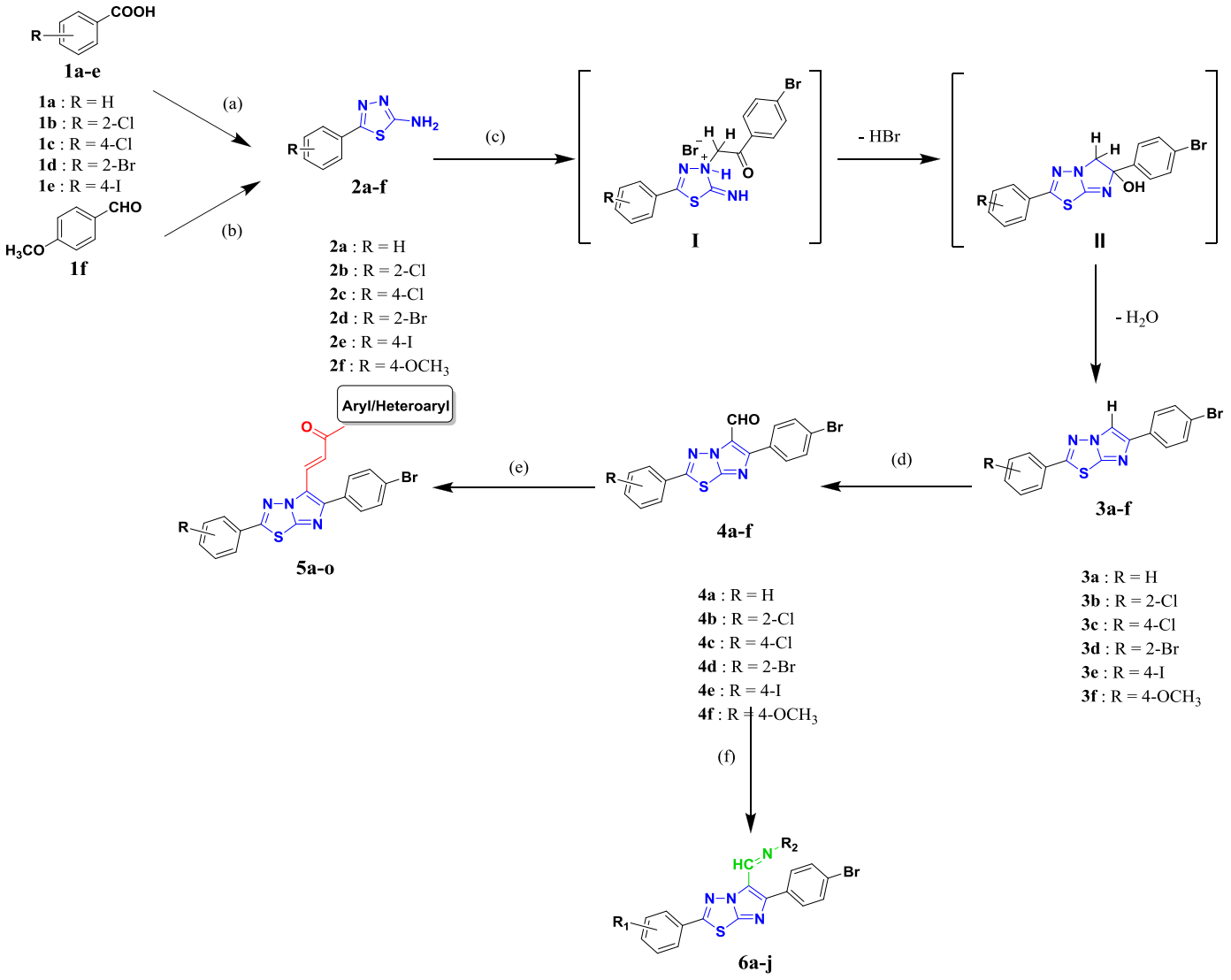


Fig. 1. Design of various chalcone (**5a**–**o**) and Schiff base (**6a**–**j**) analogs of imidazo[2,1-*b*]-1,3,4-thiadiazole by molecular hybridization approach.

series, compound **5n** (MIC = 3.125 µg/mL) exhibited good antifungal activity against clinical isolate of *C. neoformans*, while compounds **5a**, **5i** and **5k** displayed moderate activity with MIC of 6.25 µg/mL. In general, *para* and *meta* substitution on ring A with electron withdrawing (bromo and chloro), electron donating (methoxy) groups and presence of *para* bromo group on the phenyl group of Ring B were observed to be beneficial feature for the antifungal activity. In case of Chalcones, Ring C was tolerated to be unsubstituted phenyl and *p*-methyphenyl (*p*-CH_3–C_6H_4) for antifungal activity. The presence of heteroaryl group such as thiophene at ring C was not favored for the antifungal activity. In case of Schiff bases, the presence of cyclic propyl or hexyl group exhibited good



Scheme 1. The synthetic outline for the synthesis of novel series of chalcone (**5a**–**o**) and Schiff base (**6a**–**j**) analogs of imidazo[2,1-*b*]-1,3,4-thiadiazole; Reagents and conditions: (a) Thiosemicarbazide, $POCl_3$, reflux, 4 h, basify 40% NH_4OH; (b) Thiosemicarbazide, $FeCl_3$, Sodium citrate, citric acid, reflux, 1 h, basify, 40% NH_4OH; (c) 4-Bromo phenacylbromide, DMF, heating, 12–16 h; (d) DMF, $POCl_3$, 0 °C, stir, 90 °C, 6 h, $Na_2CO_3$16 h; (e) Aryl/Heteroaryl ketones, Ethanol, 10% NaOH, stir, RT, 6–10 h; (f) Cyclopropylamine or Cyclohexylamine, glacial acetic acid MW 20–30 min at 150 W.

antifungal activity. From literature, imidazo[2,1-*b*]-1,3,4-thiadiazoles derivatives were reported for the antibacterial and anti-mycobacterial activity [13,33,34]. Hence, the synthesized compounds were evaluated against Gram positive; *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC605 and Gram negative; *Escherichia coli* ATCC35218, *Pseudomonas aeruginosa* ATCC27853] bacterial strain and *Mycobacterium tuberculosis* H_{37}Rv strain. The antituburcular activity of these compounds were carried out at National Institute of Allergy and Infectious Diseases (NIAID) screening program, Bethesda, MD, USA [29–32]. All the synthesized compounds displayed moderate or no activity against bacterial and mycobacterial strain as depicted in Table 1.

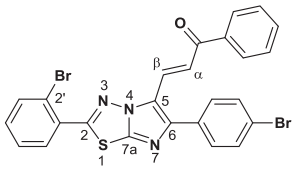
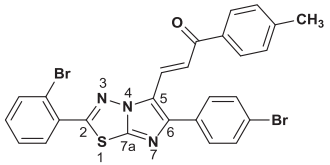
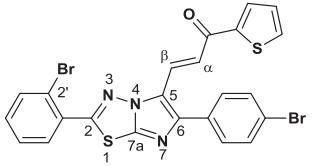
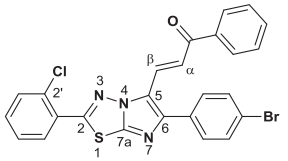
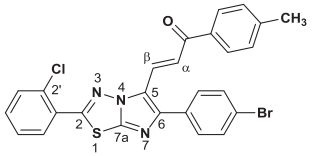
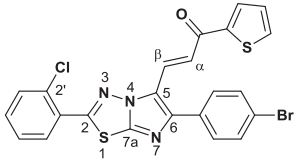
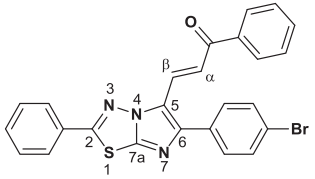
4. Conclusion

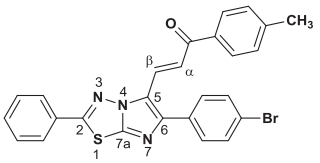
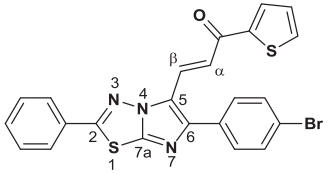
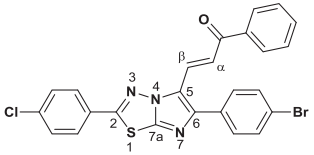
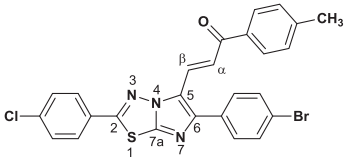
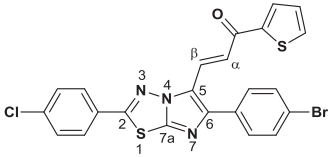
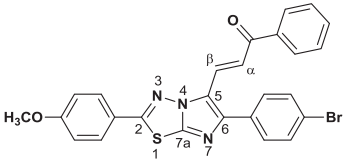
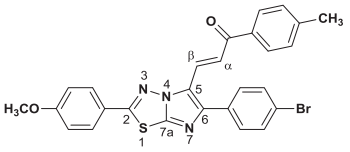
In conclusion, twenty five novel hybrids including chalcones (**5a**–**o**) and Schiff bases (**6a**–**j**) of imidazo[2,1-*b*]-1,3,4-thiadiazole scaffold were synthesized and evaluated for their antifungal, antitubercular and antibacterial activity. These synthesized hybrids displayed promising activity against tested fungal strains, in particular for both the normal and clinical isolate of *C. neoformans*. The chalcone (**5a**–**o**) derivatives exhibited significant antifungal activity when compared to the Schiff bases (**6a**–**j**). The antifungal activity displayed by compounds **5a**, **5b** and **5n** against *C. neoformans* indicates that these substituted hybrids can act as leads and can be further exploited to develop potential antifungal agents. In addition, these active chalcones of imidazothiadiazole (**5a**–**o**) also displayed moderate activity (MIC >20 μg/mL) against *M. tuberculosis* H_{37}Rv. The encouraging antifungal and anti-mycobacterial activity of synthesized novel imidazo [2,1-*b*]-1,3,4-thiadiazole derivatives through modification of ring substituents and/or additional functionalization indicated the potential for further research into the development antifungal agents against the resistant clinical isolates.

5. Experimental section

The analytical grade (AR) chemicals and reagents procured from

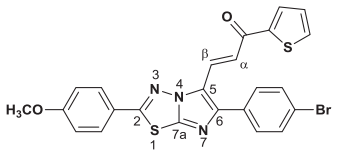
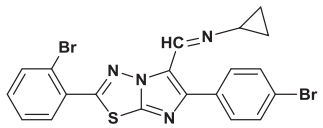
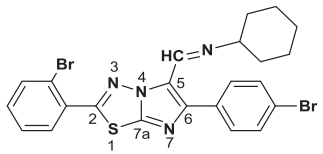
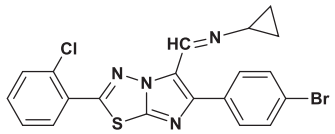
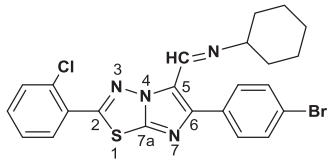
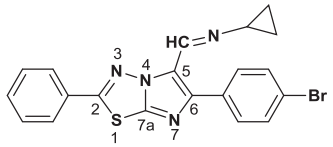
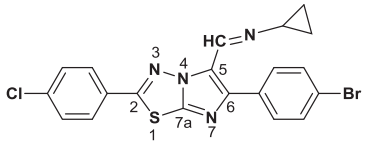
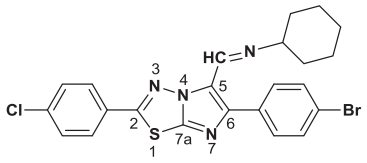
Table 1
Anti-fungal, antibacterial and anti-tubercular activity of a novel series of chalcone (**5a**–**o**) and Schiff base (**6a**–**j**) analogues of imidazo[2,1-*b*]-1,3,4thiadiazole.

Code	Structure	*C. albicans*[a] ATCC90028	*C. albicans*[a,c] (Clinical isolate)	*C. neoformans*[a] ATCC66031	*C. neoformans*[a,c] (Clinical isolate)	*A. niger*[a] ATCC16404	*S. aureus*[a] ATCC25923	*B. subtilis*[a] ATCC6051	*E. coli*[a] ATCC35218	*P. aeruginosa*[a] ATCC27853	*M. tuberculosis*[b] $H_{37}Rv$
5a		200	>200	**1.56**	**6.25**	>200	200	>200	>200	>200	>20
5b		200	>200	**1.56**	**12.5**	>200	100	>200	>200	>200	>20
5c		>200	>200	**3.125**	**12.5**	>200	>200	>200	>200	>200	>20
5d		>200	>200	12.5	12.5	>200	>200	>200	100	>200	>20
5e		50	>200	50	100	>200	200	>200	>200	>200	>20
5f		50	>200	200	200	>200	>200	>200	>200	>200	>20
5g		>200	>200	25	200	>200	>200	>200	>200	>200	>20

5h		25	>200	25	100	>200	>200	>200	>200	>200	>20
5i		200	>200	6.25	6.25	>200	>200	>200	>200	>200	>20
5j		>200	>200	6.25	12.5	>200	>200	>200	>200	>200	>20
5k		50	>200	**3.125**	**6.25**	>200	>200	200	>200	>200	>20
5l		25	>200	12.5	12.5	>200	>200	>200	>200	>200	>20
5m		50	>200	**3.125**	**12.5**	>200	>200	>200	200	>200	>20
5n		200	>200	**1.56**	**3.125**	>200	>200	>200	200	>200	>20

(*continued on next page*)

Table 1 (*continued*)

Code	Structure	*C. albicans*[a] ATCC90028	*C. albicans*[a,c] (Clinical isolate)	*C. neoformans*[a] ATCC66031	*C. neoformans*[a,c] (Clinical isolate)	*A. niger*[a] ATCC16404	*S. aureus*[a] ATCC25923	*B. subtilis*[a] ATCC6051	*E. coli*[a] ATCC35218	*P. aeruginosa*[a] ATCC27853	*M. tuberculosis*[b] $H_{37}Rv$
5o		100	>200	25	200	>200	>200	>200	>200	>200	>20
6a		25	50	100	200	100	200	>200	200	50	ND
6b		50	50	100	200	50	200	>200	200	50	ND
6c		25	50	100	>200	100	200	>200	200	50	ND
6d		12.5	25	100	>200	100	200	>200	200	50	ND
6e		25	50	100	>200	100	200	200	200	50	ND
6f		25	50	100	200	100	200	200	200	50	ND
6g		50	50	100	>200	100	200	200	200	50	ND

commercial suppliers (Merck and Sigma–Aldrich) and used without further purification. The solvents except AR grade were purified as per the literature methods when necessary. The progress of the reactions and the purity of the synthesized compounds were monitored by thin layer chromatography using pre-coated silica gel plates (Merck), UV light and/or Iodine vapors were used as visualization agents. Melting points were determined in open capillaries using (Electrothermal 9300) digital melting point apparatus and were uncorrected. The IR spectra were recorded on (Perkin Elmer 100) FT-IR spectrophotometer with universal ATR sampling accessory. ^{1}H and ^{13}C NMR spectra were recorded on (Bruker Advance IV) NMR spectrometer at 400 and 100 MHz respectively using $CDCl_3$ and DMSO-d_6. (CEM *Discover*, *Explorer*-12 Hybrid) Microwave reactor was used to synthesize some Schiff base derivatives.

5.1. General procedure for the synthesis of 2-amino-5-substitutedphenyl 1,3,4-thiadiazole (**2a**–**f**)

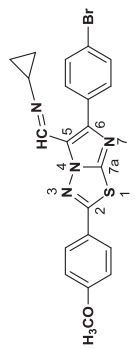
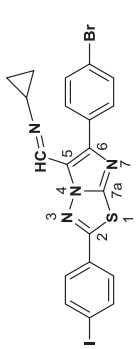
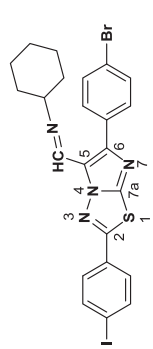
The each substituted benzoic acid (6.001 g, 0.05 mol), thiosemicarbazide (4.557 g, 0.05 mol) and $POCl_3$ (13 ml) were thoroughly stirred, mixed and heated at 75 °C for 1 h with constant stirring. After cooling to rt, water (40 ml) was slowly added. The reaction mixture was further refluxed for 4 h. After cooling, the mixture was basified to pH 8 by careful drop wise addition of 10% aqueous ammonia solution with constant stirring. The precipitate thus obtained was filtered and recrystallized from ethanol: water mixture to yield the pure compounds (**2a**–**e**) [13].

5.2. General procedure for the synthesis of 2-substitutedphenyl-6-(4-bromophenyl) imidazo(2,1-b)1,3,4-thiadiazole (**3a**–**f**)

A mixture of equimolar quantities of 2-amino-5-substitutedphenyl 1,3,4-thiadiazole (2 g, 0.0078 mol) and 4-bromo phenacylbromide (2.1 g, 0.0078 mol) was heated for 12–16 h with constant stirring in DMF (10 ml). The reaction mixture was poured onto crushed ice and the solid hydrobromide separated was filtered, washed and dried. Neutralization of hydrobromide salt intermediate with cold aqueous solution of sodium carbonate yielded the corresponding free bases (**3a**–**f**), which were further purified by recrystallization from ethanol [11].

5.3. General procedure for the synthesis of 2-substitutedphenyl-5-formyl-6-(4-bromophenyl) imidazo(2,1-b)1,3,4-thiadiazole (**4a**–**f**)

Vilsmeier–Haack reagent was freshly prepared by the careful addition of phosphoryl chloride (2 ml, 0.021 mol) in DMF (8 ml, 0.103 mol) at 0 °C with constant stirring. Then an appropriately 2-substitutedphenyl-6-(4-bromophenyl)imidazo(2,1-*b*)1,3,4-thiadiazole (2 g, 0.0045 mol) was added to the reagent with continuous stirring maintaining the temperature at 0 °C for initial 30 min and later stirred at rt for 2 h, and finally at 60 °C for another 2 h. The reaction mixture was then poured in sodium carbonate solution and stirring was continued at 90 °C for 2 h. After cooling to rt, the reaction mixture was suspended into water, extracted with (20 ml) dichloromethane (3 times), and the collective extracts were washed with water and dried over anhydrous sodium sulfate. The residue obtained after the in-vacuo removal of dichloromethane was further recrystallized from ethanol to afford the desired compound (**4a**–**f**) as colorless crystalline solid [35].

6h		25	50	100	>200	100	200	200	200	25	ND					
6i		25	50	100	>200	100	200	200	200	50	ND					
6j		25	50	100	>200	100	200	200	200	50	ND					
	Amphotericin B	**0.25**	**25**	**1–2**	**1–2**	**1.95**	–	–	–	–	–					
	Moxcillin	–	–	–	–	–	**<0.39**	**<0.39**	**<0.39**	**<0.39**	–					
	Rifampicin	–	–	–	–	–	–	–	–	–	0.0067					

The bold values represents the best activity/most active compounds against that particular strain compared to other synthesized compounds.

[a] MIC in μg/mL.

[b] MIC in μM/mL.

[c] Well characterized stored clinical isolates obtained from department of microbiology, Inkosi Albert Luthuli hospital, Durban, South Africa.

5.4. General procedure for the synthesis of 3-(2-(2-substitutedphenyl)-6-(4-bromophenyl)imidazo[2,1-b][1,3,4] thiadiazol-5-yl)-1-substituted aryl/heteroaryl-prop-2-en-1-one (**5a**–**o**)

In a round bottom flask equipped with sealed mechanical stirrer, 10% sodium hydroxide solution (2 ml) and (10 ml) ethanol were constantly stirred in an ice-bath for 2 min. Then, the appropriately substituted aryl/heteroaryl ketones (0.3 g, 0.002 mol) and compounds (**4a**–**f**) (1 g, 0.002 mol) were slowly added to the above mixture and stirred for 30 min. The reaction mixture was further stirred at rt for 6–10 h. The solid precipitate obtained was filtered, dried and recrystallized using ethanol to yield the chalcone derivatives (**5a**–**o**) of imidazo [2,1-*b*][1,3,4]thiadiazoles [27].

5.4.1. 3-(2-(2-bromophenyl)-6-(4-bromophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1 phenylprop-2-en-1-one **5a**

Yellow crystals; Yield 79%, mp. 231–233 °C; IR [ATR, ν_{max}, cm^{-1}]: 3054.1(Ar C–H), 3001.4 (C=C–H), 1723.8 (C=N), 1661.3 (C=O), 1590 (C=C), 687.2 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.37–8.33 (d, J = 15 Hz, 1H, H β), 8.09–8.07 (d, J = 15 Hz, 1H, Hα), 8.06–8.05 (m, 1H, Ar–H), 7.92–7.90 (m, 1H, Ar–H), 7.85–7.83 (m, 1H, Ar–H), 7.69–7.54 (m, 4H, Ar–H), 7.52–7.46 (m, 6H, Ar–H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 189.9, 160.3, 150.0, 149.4, 138.3, 134.8, 132.8, 132.6, 132.3, 132.1, 132.1, 131.8, 130.3, 130.2, 129.7, 129.7, 128.8, 128.6, 128.5, 128.2, 128.1, 123.2, 122.1, 121.5, 120.2; HRMS (EI) m/z calcd for $C_{25}H_{15}Br_2N_3OS$: 562.9303; found: 562.9308.

5.4.2. 3-(2-(2-bromophenyl)-6-(4-bromophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1-p-tolylprop-2-en-1-one **5b**

Yellow crystals; Yield 56%, mp. 225–227 °C; IR [ATR, ν_{max}, cm^{-1}]: 3057.8 (Ar C–H), 2923.8 (C=C–H), 1728 (C=N), 1658.9 (C=O), 1587.3 (C=C), 687.2 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.37–8.33 (d, J = 15 Hz, 1H, H β), 8.08–8.04 (d, J = 15, 1H, H α), 8.01–7.99 (m, 2H, Ar–H), 7.92–7.83 (m, 2H, Ar–H), 7.69–7.63 (m, 4H, Ar–H), 7.56–7.44 (m, 2H, Ar–H), 7.31–7.29 (m, 2H, Ar–H), 2.43 (s, 3H, Ar–CH_3); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 189.4, 160.2, 149.8, 149.3, 143.6, 135.7, 134.8, 132.5, 132.4, 132.1, 131.8, 130.5, 130.3, 129.1, 129.6, 129.3, 129.1, 129.1, 128.6, 128.1, 127.8, 123.2, 122.1, 121.5, 120.4, 21.6; HRMS (EI) m/z calcd for $C_{26}H_{17}Br_2N_3OS$: 576.9459; found: 576.9463.

5.4.3. 3-(2-(2-bromophenyl)-6-(4-bromophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1-(thiophen-2-yl)prop-2-en-1-one **5c**

Yellow crystals; Yield 53%, mp. 263–265 °C; IR [ATR, ν_{max}, cm^{-1}]: 3064.6 (Ar C–H), 2962.7 (C=C–H), 1724.4 (C=N), 1647.3 (C=O), 1578.6 (C=C), 707.3(C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.21–8.17 (d, J = 15 Hz, 1H, H β), 8.06–8.02 (d, J = 15, 1H, Hα), 8.89–7.61 (m, 8H, Ar–H), 7.56–7.52 (m, 1H, thiophene 3H), 7.47–7.42 (m, 1H, thiophene 4H), 7.17–7.15 (m, 1H, thiophene 5H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 181.9, 160.3, 150.1, 149.4, 145.9, 134.8, 134.1, 133.7, 132.6, 132.3, 132.1, 131.8, 131.7, 130.3, 130.2, 129.7, 128.2, 128.1, 127.5, 123.3, 122.1, 121.3, 120.1; HRMS (EI) m/z calcd for $C_{23}H_{13}Br_2N_3OS_2$: 568.8867; found: 568.8871.

5.4.4. 3-(6-(4-bromophenyl)-2-(2-chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1-phenylprop-2-en-1-one **5d**

Yellow crystals; Yield 62%, mp. 223–225 °C; IR [ATR, ν_{max}, cm^{-1}]: 3064.5(Ar C–H), 2601.3 (C=C–H), 1714.6 (C=N), 1654.7 (C=O), 1586 (C=C), 687.5 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.34–8.30 (d, J = 16 Hz, 1H, H β), 8.07–8.05 (d, J = 15, 1H, H α), 8.04–7.98 (m, 3H, Ar–H), 7.66–7.52 (m, 5H, Ar–H), 7.51–7.46 (m, 5H, Ar–H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 189.9, 159.1, 150.1, 149.5, 138.3, 132.8, 132.8, 132.7, 132.4, 132.1, 132.1, 131.4, 131, 131, 130.2, 128.6, 128.6, 128.4, 128.2, 128.2, 127.6, 124.5, 123.2, 121.3, 120.2; HRMS (EI) m/z calcd for $C_{25}H_{15}BrClN_3OS$: 518.9808; found: 518.9812.

5.4.5. 3-(6-(4-bromophenyl)-2-(2-chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1- p-tolylprop-2-en-1-one **5e**

Yellow crystals; Yield 78%, mp. 236–238 °C; IR [ATR, ν_{max}, cm^{-1}]: 3054.6 (Ar C–H),2599.9 (C=C–H), 1731.4 (C=N), 1658.6 (C=O), 1587.7 (C=C), 756.9 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.34–8.30 (d, J = 16 Hz, 1H, H β), 8.06–8.05 (d, J = 15, 1H, H α), 8.03–7.98 (m, 3H, Ar–H), 7.66–7.60 (m, 5H, Ar–H),7.52–7.48 (m, 2H, Ar–H), 7.30–7.28 (d, 2H, Ar–H), 2.42 (s, 3H, Ar–CH3); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 189.4, 159.0, 149.9, 149.4, 143.6, 135.8, 132.4, 132.1, 132.1, 131.4, 131, 131, 130.1, 130.1, 129.3, 129.3, 128.6, 128.6, 127.8, 127.8, 127.6, 127.6, 123.2, 121.4, 120.3. 21.7; HRMS (EI) m/z calcd for $C_{26}H_{17}BrClN_3OS$: 532.9964; found: 532.9968.

5.4.6. 3-(6-(4-bromophenyl)-2-(2-chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1-(thiophen-2-yl)prop-2-en-1-one **5f**

Yellow crystals; Yield 86%, mp. 260–263 °C; IR [ATR, ν_{max}, cm^{-1}]: 3062.4(Ar C–H), 2585.1 (C=C–H), 1899.3 (C=N), 1646.9 (C=O), 1584.5 (C=C), 725.8 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.23–8.19 (d, J = 15 Hz, 1H, H β),8.09–8.07 (d, J = 15, 1H, Hα), 8.06–8.04 (m, 1H, Ar–H), 7.89–7.88 (m, 1H, Ar–H),7.67–7.63 (m, 1H, Ar–H), 7.55–7.52 (m, 2H, thiophene 3H,4H), 7.20–7.18 (m, 1H thiophene, 5H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 181.9, 159.1, 150.2, 145.8, 133.6, 132.8, 132.4, 132.1, 131.8, 131.6, 131.4, 131, 130.2, 129.7, 129.7, 128.2, 128.2, 127.6, 127.5, 125, 123.3, 121.1, 120.1; HRMS (EI) m/z calcd for $C_{23}H_{13}BrClN_3OS_2$: 524.9372; found: 524.9376.

5.4.7. 3-(6-(4-bromophenyl)-2-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1-phenylprop-2-en-1-one **5g**

Yellow crystals; Yield 53%, mp. 263–265 °C; IR [ATR, ν_{max}, cm^{-1}]: 3064 (Ar C–H), 1655.3 (C=O), 1572.5 (C=C), 690 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.29–8.25 (d, J = 15 Hz, 1H, H β), 8.03–8.00 (d, J = 15, 1H, Hα), 7.96–7.89 (m, 4H, Ar–H), 7.60–7.56 (m, 5H, Ar–H), 7.51–7.40 (m, 5H, Ar–H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 190.0, 162.7, 149.8, 148.6, 138.4, 132.7, 132.2, 132.2, 132.2, 132.1, 132.1, 130.1, 130.1, 130.1, 129.5, 129.5, 128.7, 128.7, 128.4, 128.4, 128.3, 126.9, 123.1, 121.6, 120.3; HRMS (EI) m/z calcd for $C_{25}H_{16}BrN_3OS$: 485.0197; found: 485.0201.

5.4.8. 3-(6-(4-bromophenyl)-2-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1-p-tolylprop-2-en-1-one **5h**

Yellow crystals; Yield 80%, mp. 244–246 °C; IR [ATR, ν_{max}, cm^{-1}]: 3033.1 (Ar C–H), 1655.6 (C=O), 1582.9 (C=C), 688.8 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.38–8.34 (d, J = 15 Hz, 1H, H β),8.08–8.04 (d, J = 15, 1H, Hα), 8.02–7.92 4 (m, 4H, Ar–H), 7.67–7.66 (m, 3H, Ar–H), 7.62–7.60 (m, 4H, Ar–H), 7.37–7.35 (m, 2H, Ar–H), 2.48 (s, 3H, Ar-CH3); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 189.5, 162.6, 148.5, 143.6, 135.8, 132, 132, 131.9, 131.9, 130.5, 130.5, 130.1, 130.1, 129.5, 129.46, 129.41, 128.6, 128.6, 127.9, 127.9, 126.9, 126.9, 123.1, 121.6, 120.5, 21.7; HRMS (EI) m/z calcd for $C_{26}H_{18}BrN_3OS$: 499.0354; found: 499.0358.

5.4.9. 3-(6-(4-bromophenyl)-2-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1-(thiophen-2-yl)prop-2-en-1-one **5i**

Yellow crystals; Yield 66%, mp. 267–269 °C; IR [ATR, ν_{max}, cm^{-1}]: 3074.7 (Ar C–H), 1642.6 (C=O), 1575.6 (C=C), 685.3 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.21–8.17 (d, J = 15 Hz, 1H, H β), 8.04–8.01 (d, J = 15, 1H, H α), 7.97–7.95 (m, 2H, Ar–H), 7.90–7.88 (m, 1H, Ar–H),7.69–7.68 (m, 1H, Ar–H), 7.62–7.59 (m, 3H, Ar–H), 7.58–7.57 (m, 2H, thiophene 3H,4H), 7.22–7.20 (m, 1H, thiophene 5H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 181.8, 162.7, 149.8, 148.6, 145.8, 133.6, 132.3, 132.3, 132.2, 132.2, 132.1, 131.5, 131.5, 130.1, 130.1,

129.8, 129.5, 129.5, 128.3, 127.6, 126.9, 123.2, 121.4; HRMS (EI) *m/z* calcd for $C_{23}H_{14}BrN_3OS_2$: 490.9762; found: 490.9766.

5.4.10. 3-(6-(4-bromophenyl)-2-(4-chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1-phenylprop-2-en-1-one ***5j***

Yellow crystals; Yield 64%, mp. 247–250 °C; IR [ATR, ν_{max}, cm^{-1}]: 3063.5 (Ar C–H), 1656.9 (C=O), 1572.6 (C=C), 679.3 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.29–8.25 (d, *J* = 15 Hz, 1H, H β), 8.07–8.05 (d, *J* = 15, 1H, H α), 8.04–8.00 (m, 2H, Ar–H), 7.90–7.88 (d, *J* = 8.56 Hz, 2H, Ar–H), 7.62–7.56 (m, 5H, Ar–H), 7.54–7.50 (m, 4H, Ar–H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 190, 161.4, 149.9, 148.7, 138.5, 138.3, 132.8, 132.8, 132.2, 132.2, 132.1, 132.1, 130.1, 129.9, 129.9, 128.9, 128.9, 128.7, 128.7, 128.4, 128.3, 128.1, 123.2, 121.6, 120.5; HRMS (EI) *m/z* calcd for $C_{25}H_{15}BrClN_3OS$: 518.9808; found: 518.9812.

5.4.11. 3-(6-(4-bromophenyl)-2-(4-chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1-p-tolylprop-2-en-1-one ***5k***

Yellow crystals; Yield 50%, mp. 246–248 °C; IR [ATR, ν_{max}, cm^{-1}]: 2915.3 (Ar C–H), 1658.5 (C=O), 1586.7 (C=C), 810.2 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.29–8.25 (d, *J* = 15 Hz, 1H, H β), 8.03–7.99 (d, *J* = 15, 1H, Hα), 7.98–7.96 (d, *J* = 8.12 Hz, 2H, Ar–H), 7.90–7.87 (d, *J* = 8.60 Hz, 2H, Ar–H), 7.62–7.60 (m, 4H, Ar–H), 7.56–7.54 (d, *J* = 8.48 Hz, 2H, Ar–H), 7.33–7.31 (d, *J* = 8.08 Hz, 2H, Ar–H), 2.44 (s, 3H, Ar-CH3); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 189.5, 161.3, 149.6, 148.2, 143.7, 138.4, 135.8, 132.3, 132.1, 132.1, 131.8, 130.1, 130.1, 129.8, 129.4, 129.4, 129.2, 129, 128.5, 128.3, 128. 127.8, 123.1, 121.6, 120.6, 21.6; HRMS (EI) *m/z* calcd for $C_{26}H_{17}BrClN_3OS$: 532.9964; found: 532.9968.

5.4.12. 3-(6-(4-bromophenyl)-2-(4-chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1-(thiophen-2-yl)prop-2-en-1-one ***5l***

Yellow crystals; Yield 45%, mp. 266–268 °C; IR [ATR, ν_{max}, cm^{-1}]: 3341.2 (Ar C–H), 1645.4 (C=O), 1406 (C=C), 719.2 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.17–8.13 (d, *J* = 15 Hz, 1H, H β), 8.04–8.00 (d, *J* = 15, 1H, Hα), 7.91–7.87 (m, 3H, Ar–H), 7.69–7.62 (m, 5H, Ar–H), 7.59–7.55 (m, 2H, thiophene 3H,4H), 7.22–7.20 (t, *J* = 8.68 Hz, 1H, thiophene 5H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 181.8, 161.4, 149.9, 148.4, 145.7, 138.5, 133.6, 132.2, 132.1, 132.1, 131.8, 131.5, 130.1, 129.9, 129.5, 129.3, 128.3, 128, 127.9, 127.5, 123.3, 121.44, 120.4; HRMS (EI) *m/z* calcd for $C_{23}H_{13}BrClN_3OS_2$: 524.9372; found: 524.9376.

5.4.13. 3-(6-(4-bromophenyl)-2-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1-phenylprop-2-en-1-one ***5m***

Pale Yellow crystals; Yield 52%, mp. 229–231 °C; IR [ATR, ν_{max}, cm^{-1}]: 2964.7 (Ar C–H), 1652.6 (C=O), 1563.8 (C=C), 818.8 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.32–8.28 (d, *J* = 15 Hz, 1H, H β), 8.08–8.04 (m, 2H, Ar–H), 8.04–8.00 (d, *J* = 15, 1H, H α), 7.89–7.87 (d, *J* = 8.88 Hz, 2H, Ar–H), 7.64–7.50 (m, 7H, Ar–H), 7.06–7.03 (d, *J* = 8.84 Hz, 2H, Ar–H), 3.92 (s, 3H, Ar-OCH3); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 190.1, 162.8, 162.5, 149.5, 148.5, 138.4, 132.7, 132.4, 132, 132, 130.1, 130.1, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 123, 122.3, 121.5, 120.1, 114.9, 55.6; HRMS (EI) *m/z* calcd for $C_{26}H_{18}BrN_3O_2S$: 515.0303; found: 515.0308.

5.4.14. 3-(6-(4-bromophenyl)-2-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1-p-tolylprop-2-en-1-one ***5n***

Yellow crystals; Yield 72%, mp. 252–254 °C; IR [ATR, ν_{max}, cm^{-1}]: 2939.2 (Ar C–H), 1654.6 (C=O), 1581.9 (C=C), 764.3 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.32–8.28 (d, *J* = 15 Hz, 1H, H β), 8.03–8.00 (d, *J* = 13 Hz, 1H, H α), 7.99–7.98 (d, *J* = 5.76 Hz, 2H, Ar–H), 7.89–7.87 (d, *J* = 8.7 Hz, 2H, Ar–H), 7.64–7.59 (q, *J* = 4.2 Hz, 4H, Ar–H), 7.33–7.31 (d, *J* = 8.0 Hz, 2H, Ar–H), 7.06–7.04 (d, *J* = 4.84 Hz, 2H, Ar–H), 3.9 (s, 3H, Ar-OCH3), 2.46 (s, 3H, Ar-CH3); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 189.6, 162.7, 149.3, 143.5, 135.9, 132, 132, 131.7, 131.7, 130.1, 130.1, 130.1, 129.4, 129.4, 129.1, 129.1, 128.5, 128.5, 128.1, 128.1, 123, 122.4, 121.5, 120.3, 114.9, 55.6, 21.7; HRMS (EI) *m/z* calcd for $C_{27}H_{20}BrN_3O_2S$: 529.0460; found: 529.0464.

5.4.15. 3-(6-(4-bromophenyl)-2-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-1-(thiophen-2-yl)prop-2-en-1-one ***5o***

Orange crystals; Yield 57%, mp. 257–259 °C; IR [ATR, ν_{max}, cm^{-1}]: 3063.7 (Ar C–H), 1674 (C=N), 1647 (C=O), 1579.9 (C=C), 716.5 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.19–8.16 (d, *J* = 15 Hz, 1H, H β), 8.03–7.99 (d, *J* = 15 Hz, 1H, H α), 7.90–7.87 (m, 3H, Ar–H), 7.64–7.59 (m, 5H, Ar–H), 7.22–7.19 (m, 1H, thiophene 3H), 7.07–7.05 (d, *J* = 8.76 Hz, 2H, thiophene 4H,5H), 3.9 (s, 3H Ar-OCH3); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 181.9, 162.8, 162.5, 149.6, 148.6, 145.8, 133.6, 132.4, 132, 131.8, 131.5, 130.1, 129.5, 128.5, 128.4, 128.3, 127.7, 123.1, 122.3, 121.3, 120, 114.9, 114.4, 55.6; HRMS (EI) *m/z* calcd for $C_{24}H_{16}BrN_3O_2S_2$: 520.9867; found: 520.9871.

*5.5. General procedure for the synthesis of 6-(4-bromophenyl)-2-(substitutedphenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene) cyclosubstitutedamine (**6a**–**j**)*

Compounds (**4a**–**f**) (0.2 g, 0.0003 mol) and aliphatic cyclic amine (0.025 g, 0.0003 mol) in ethanol (5 ml) and catalytic amount of glacial acetic acid were transferred into a 10 mL microwave tube kitted with mechanical stirrer. The reaction mixture was irradiated with microwave radiations for 20–30 min at 150 psi pressure. The completion of reaction was monitored by TLC using ethyl acetate and hexane (1:3). The solid thus obtained was filtered, dried and recrystallized using the suitable solvent to afford the Schiff base derivatives (**6a**–**j**) of imidazo [2, 1-*b*][1,3,4]thiadiazoles.

5.5.1. 2-(2-bromophenyl)-6-(4-bromophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)cyclopropanamine ***6a***

Yellow crystals; Yield 45%, mp. 204–206 °C; IR [ATR, ν_{max}, cm^{-1}]: 3136.9 (Ar C–H), 3064.7 (C=C–H), 1561.4 (CH=N), 749.6 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.06 (s,1H,CH=N), 7.84–7.82 (m, 1H, Ar–H), 7.74–7.70 (m, 3H, Ar–H), 7.53–7.45 (d, *J* = 8.25 Hz, 2H, Ar–H), 7.43–7.39 (m, 1H, Ar–H), 7.37–7.35 (m, 1H, Ar–H), 1.71 (br s, 4H, Aliphatic-H), 1.23 (s, 1H, Aliphatic-H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 159.5, 146.5, 145.7, 134.3, 132.8, 132.2, 132.2, 132.2, 131.8, 131.7, 130.7, 127.9, 127.9, 126.7, 122, 121.5, 109.3, 29.6, 10.2, 10.2; HRMS (EI) *m/z* calcd for $C_{20}H_{14}Br_2N_4S$: 499.9306; found: 499.9310.

5.5.2. 2-(2-bromophenyl)-6-(4-bromophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5- yl)methylene)cyclohexanamine ***6b***

Orange crystals; Yield 60%, mp. 197–199 °C; IR [ATR, ν_{max}, cm^{-1}]: 3063.3 (Ar C–H), 2852.8 (C=C–H), 1639.1 (CH=N), 752.5 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.64 (s, 1H, CH=N), 7.94–7.92 (m, 3H, Ar–H), 7.74–7.72 (m, 1H, Ar–H), 7.57–7.55 (m, 2H, Ar–H), 7.39–7.37 (m, 1H, Ar–H), 1.81–1.77 (m, 4H, Aliphatic-H), 1.65–1.60 (m, 3H, Aliphatic-H), 1.38-1-30 (m, 4H, Aliphatic-H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 160.1, 147.2, 146.2, 134.3, 132.6, 132.3, 132.1, 132, 131.5, 130.7, 130.5, 130.2, 128, 127.9, 122.6, 122.1, 121.9, 70.4, 34.4, 25.6, 25.1, 24.65, 24.60; HRMS (EI) *m/z* calcd for $C_{23}H_{20}Br_2N_4S$: 541.9775; found: 541.9779.

5.5.3. 6-(4-bromophenyl)-2-(2-chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl) methylene)cyclopropanamine ***6c***

Yellow crystals; Yield 50%, mp. 189–191 °C; IR [ATR, ν_{max}, cm^{-1}]: 3136.8 (Ar C–H), 3068.2 (C=C–H), 1648.5 (CH=N), 750 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.06 (s, 1H, CH=N), 8.00–7.98 (m, 1H, Ar–H), 7.72–7.69 (d, *J* = 8.48 Hz, 2H, Ar–H), 7.53–7.45 (m, 3H,

Ar–H), 7.43–7.39 (m, 2H, Ar–H), 1.77 (s, 4H, Aliphatic-H), 1.23 (s, 1H, Aliphatic-H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 158.2, 146.7, 145.8, 132.8, 132.6, 132.1, 132.1, 132.1, 131.8, 131, 130.9, 128.6, 128.6, 127.4, 126.7, 121.5, 109.2, 29.7, 11.2, 11.2; HRMS (EI) *m/z* calcd for $C_{20}H_{14}BrClN_4S$: 455.9811; found: 455.9815.

5.5.4. 6-(4-bromophenyl)-2-(2-chlorophenyl)imidazo[2,1-b][1,3,4] thiadiazol-5-yl) methylene)cyclohexanamine ***6d***

Yellowish green crystal; Yield 70%, mp. 188–190 °C; IR [ATR, ν_{max}, cm^{-1}]: 3056.4 (Ar C–H), 2849.8 (C=C–H), 1637.9 (CH=N), 747.2 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.68 (s, 1H, CH=N), 8.14–8.00 (m, 1H, Ar–H), 7.95–7.93 (d, *J* = 8.25 Hz, 2H, Ar–H), 7.57–7.53 (m, 2H, Ar–H), 7.45–7.40 (m, 3H, Ar–H), 1.85–1.78 (m, 4H, Aliphatic-H), 1.66–1.61 (t, *J* = 11.48 Hz, 3H, Aliphatic-H), 1.39–1.34 (m, 4H, Aliphatic-H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 158.7, 147.9, 147.3, 146.2, 132.7, 132.6, 132.1, 132.1, 131.4, 131.2, 130.9, 130.2, 128.6, 128.6, 127.4, 122.6, 121.8 70.4, 34.4, 25.7, 25.7, 25.7, 24.5; HRMS (EI) *m/z* calcd for $C_{23}H_{20}BrClN_4S$: 498.0281; found 498.0285.

5.5.5. 6-(4-bromophenyl)-2-phenylimidazo[2,1-b][1,3,4]thiadiazol-5- yl)methylene) cyclopropanamine ***6e***

Yellowish green crystals; Yield 83%, mp. 190–192 °C; IR [ATR, ν_{max}, cm^{-1}]: 3117.5 (Ar C–H), 2923 (C=C–H), 1671.4 (CH=N), 760.1 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.02 (s, 1H, CH=N), 7.87–7.84 (d, *J* = 8.40 Hz, 2H, Ar–H), 7.70–7.68 (d, *J* = 8.56 Hz, 2H, Ar–H), 7.52–7.49 (m, 5H, Ar–H), 1.64 (br s, 3H, Aliphatic-H), 1.23–120 (m, 2H, Aliphatic-H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 161.7, 145.5, 145.4, 132.8, 131.8, 131.8, 131.7, 131.7, 130.1, 130.1, 129.3, 129.3, 129.3, 126.7, 126.6, 121.4, 109.5, 32.5, 8.1, 8.1; HRMS (EI) *m/z* calcd for $C_{20}H_{15}BrN_4S$: 422.0201; found 422.0205.

5.5.6. 6-(4-bromophenyl)-2-(4-chlorophenyl)imidazo[2,1-b][1,3,4] thiadiazol-5- yl)methylene) cyclopropanamine ***6f***

Yellow crystals; Yield 52%, mp. 187–189 °C; IR [ATR, ν_{max}, cm^{-1}]: 3056 (Ar C–H), 2847.6 (C=C–H), 1640.3 (CH=N), 761.3(C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.01 (s, 1H, CH=N), 7.80–7.78 (d, *J* = 8.60 Hz, 2H, Ar–H), 7.69–7.67 (d, *J* = 8.56 Hz, 2H, Ar–H), 7.52–7.46 (m, 4H, Ar–H), 1.61 (br s, 4H, Aliphatic-H), 1.22 (s, 1H, Aliphatic-H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 160.4, 145.7, 145.3, 137.9, 132.6, 131.8, 131.8, 131.6, 129.6, 129.6, 128.6, 128.6, 127.9, 127.9, 126.6, 121.5, 109.5, 50.8, 30.9, 30.9; HRMS (EI) *m/z* calcd for $C_{20}H_{14}BrClN_4S$: 455.9811; found 455.9815.

5.5.7. 6-(4-bromophenyl)-2-(4-chlorophenyl)imidazo[2,1-b][1,3,4] thiadiazol-5-yl)methylene) cyclohexanamine ***6g***

Yellowish green crystal; Yield 61%, mp. 238–240 °C; IR [ATR, ν_{max}, cm^{-1}]: 3147.2 (Ar C–H), 2848.3 (C=C–H), 1652 (CH=N), 730.9(C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.62 (s, 1H, CH=N), 7.93–7.90 (d, *J* = 8.64 Hz, 2H, Ar–H), 7.86–7.84 (d, *J* = 8.52 Hz, 2H, Ar–H), 7.57–7.55 (d, *J* = 8.56 Hz, 2H, Ar–H), 7.48–7.46 (d, *J* = 8.52 Hz, 2H, Ar–H), 1.83–1.79 (m, 4H, Aliphatic-H), 1.67–1.62 (m, 3H, Aliphatic-H), 1.41–1.30 (m, 4H, Aliphatic-H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 161, 147.1, 146.2, 138, 132.5, 132, 131.5, 131.1, 130.5, 130.1, 129.8, 129.1, 128.6, 128.3, 127.7, 122.68, 122.2, 70.4, 34.4, 25.7, 25.7, 25.7, 24.5; HRMS (EI) *m/z* calcd for $C_{23}H_{20}BrClN_4S$: 498.0281; found 498.0285.

5.5.8. 6-(4-bromophenyl)-2-(4-methoxyphenyl)imidazo[2,1-b] [1,3,4]thiadiazol-5-yl)methyl yl)methylene)cyclopropanamine ***6h***

Yellowish green crystal; Yield 62%, mp. 201–203 °C; IR [ATR, ν_{max}, cm^{-1}]: 3008.4 (Ar C–H), 2934.1 (C=C–H), 1605.4 (CH=N), 722 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 7.97 (s, 1H, CH=N), 7.96–7.77 (d, *J* = 8.80 Hz, 2H, Ar–H), 7.68–7.66 (d, *J* = 8.44 Hz, 2H, Ar–H), 7.51–7.49 (d, *J* = 8.52 Hz, 2H, Ar–H), 6.99–6.96 (d, *J* = 8.76 Hz, 2H, Ar–H), 3.86 (s, 3H, Ar-OCH_3), 2.14 (s, 1H, Aliphatic-H), 1.80 (br s, 4H, Aliphatic-H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 162.4, 161.6, 145.3, 145.1, 141.7, 133.9, 132.9, 131.8, 131.6, 129.2, 128.3, 126.5, 122.7, 121.2, 114.7, 114.5, 109.4, 55.5. 34.2, 10.6, 10.6; HRMS (EI) *m/z* calcd for $C_{21}H_{17}BrN_4OS$: 452.0306; found 452.0310.

5.5.9. 6-(4-bromophenyl)-2-(4-iodophenyl)imidazo[2,1-b][1,3,4] thiadiazol-5-yl)methylene)cyclopropanamine ***6i***

Yellowish green crystal; Yield 57%, mp. 203–205 °C; IR [ATR, ν_{max}, cm^{-1}]: 3138.8 (Ar C–H), 2961.2 (C=C–H), 1581.2 (CH=N), 729.8 (C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.00 (s, 1H, CH=N), 7.85–7.83 (d, *J* = 8.48 Hz, 2H, Ar–H), 7.69–7.67 (d, *J* = 8.48 Hz, 2H, Ar–H), 7.58–7.56 (d, *J* = 8.48 Hz, 2H, Ar–H),7.52–7.50 (d, *J* = 8.48 Hz, 2H, Ar–H), 1.76 (br s, 4H, Aliphatic-H)1.23 (s, 1H, Aliphatic-H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 160.7, 145.7, 145.2, 138.5, 138.5, 132.6, 131.8, 131.8, 131.6, 129.6, 129.1, 129.1, 128, 126.6, 121.5, 109.5, 98.3, 30.9, 10.3, 10.3; HRMS (EI) *m/z* calcd for $C_{20}H_{14}BrIN_4S$: 547.9167; found 547.9171.

5.5.10. 6-(4-bromophenyl)-2-(4-iodophenyl)imidazo[2,1-b][1,3,4] thiadiazol-5-yl)methylene)cyclohexanamine ***6j***

Pale green crystal; Yield 44%, mp. 239–241 °C; IR [ATR, ν_{max}, cm^{-1}]: 2918.3 (Ar C–H), 2848.3 (C=C–H), 1635.6 (CH=N), 764.7(C–Br); ^{1}H NMR [400 MHz, $CDCl_3$, δ ppm]: 8.62 (s, 1H, CH=N), 7.93–7.91 (d, *J* = 8.48 Hz, 2H, Ar–H), 7.84–7.82 (d, *J* = 8.48 Hz, 2H, Ar–H), 7.61–7.60 (d, *J* = 8.42 Hz, 2H, Ar–H), 7.56–7.54 (2H, Ar–H), 1.86–1.79 (m, 4H, Aliphatic-H) 1.67–1.62 (m, 3H, Aliphatic-H) 1.41–1.21 (m, 4H, Aliphatic-H); ^{13}C NMR [101 MHz, $CDCl_3$, δ ppm]: 161.2, 147, 146.4, 145.2, 138.5, 138.4, 132.5, 132, 131.4, 130.4, 130.1, 129.5, 128.3, 128.2, 122.6, 122.2, 98.4, 70.4, 34.4, 30.9, 29.7, 25.7, 24.5; HRMS (EI) *m/z* calcd for $C_{23}H_{20}BrIN_4S$: 589.9637; found 589.9641.

5.6. Biological activity

5.6.1. In vitro evaluation of antimicrobial activity

The chalcone (**5a**–**o**) and Schiff base (**6a**–**j**) derivatives of imidazo[2,1-*b*]- 1,3,4 thiadiazole were further assessed for antimicrobial activity against panel of bacterial and fungal strains by following earlier reported MIC assay method using resazurin dye [36–38].

5.6.2. Microorganism used

Standard cultures of two gram + ve [*S. aureus* ATCC25923, *B. subtilis* ATCC6051], two gram –ve [*E. coli* ATCC35218, *P. aeruginosa* ATCC27853], three fungal strains [*C. albicans* ATCC90028, *C. neoformans* ATCC66031 and *A. niger* ATCC16404] and two clinical isolates of [*C. albicans* and *C. neoformans*] were used for the antibacterial and antifungal activity respectively. Culturing and subculturing (one day prior to testing) of these microorganisms was carried out at the department of microbiology, Inkosi Albert Luthuli hospital, Durban, South Africa. Subcultering of these microorganisms were used in this assay.

5.6.3. In vitro evaluation of antitubercular activity

The Anti-TB activity of the synthesized compounds was determined by measuring bacterial growth after 5 d in the presence of test compounds. Compounds were prepared as 10-point two-fold serial dilutions in DMSO and diluted into 7H9-Tw-OADC medium in 96-well plates with a final DMSO concentration of 2%. The highest concentration of compound was 200 μM where compounds were soluble in DMSO at 10 mM. For compounds with limited solubility, the highest concentration was 50× less than the stock concentration *e.g.* 100 μM for 5 mM DMSO stock, 20 μM for 1 mM DMSO stock. For potent compounds, assays were repeated at lower

starting concentrations. Each plate included assay controls for background (medium/DMSO only, no bacterial cells), zero growth (100 μM rifampicin) and maximum growth (DMSO only), as well as a rifampicin dose response curve. Plates were inoculated with *M. tuberculosis* and incubated for 5 days: growth was measured by OD_{590} and fluorescence (Ex 560/Em 590) using a BioTek™ Synergy 4 plate reader. Growth was calculated separately for OD_{590} and RFU [39–41].

Acknowledgment

The authors sincerely thank College of Health Science, University of KwaZulu-Natal, Durban, South Africa (CS78) and the National Research Foundation (NRF) for funding this project. We are also grateful to National Health laboratory services (NHLS), Inkosi Albert Luthuli Central Hospital (Durban, South Africa) for performing the antimicrobial activity as well as the Infectious Disease Research Institute (IDRI), National Institute of Allergy and Infectious Diseases (NIAID) screening program, Bethesda, MD, USA for carrying out the antitubercular testing. The authors would also like to acknowledge Mr. Dilip Jagjivan (School of Chemistry and Physics, UKZN, South Africa) for NMR spectral data.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.03.021.

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