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Research Article

Synthesis and Anti-leishmanial Activity Evaluation of Some 2,3-Disubstituted Quinazoline-4(3H)-Ones Bearing Quinoline and Pyrazole Moieties

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Abstract

Based on the progress reports of biologically active hetrocycles; 2,3-disubstituted-quinazoline-4(3H)-ones, quinolines and pyrazoles are among important scaffolds with promising anti-leishmanial activity. Despite to those findings, demand of new molecules with clinically applicable biological activity to halt leishmaniasis is still an active research area. This present investigation has attempted the synthesis of new hybrid scaffolds of 2,3-disubstituted-quinazoline-4(3H)-one pharmacophore bearing biologically active quinoline and pyrazole moieties and evaluate their enhanced anti-leishmanial activity. Upon simple condensation and cyclization reactions of the essential intermediates; 3-aryl-2-methyl-quinazolin-4-(3H)-ones with some pyrazolyl-4-carboxaldehydes and quinoline-3-carbaldehyde; new hybrids of 2-pyrazolyl-quinazoline-4(3H)-ones (4a-c) and 3-aryl-2-quinolinyl-quinazoline-4(3H)-ones (5a-c) were synthesized. Structures for the synthesized compounds were determined using elemental microanalysis, IR, 1H NMR and 13C NMR. *In vitro* anti-leishmanial activities of the synthesized compounds were evaluated using L. *donovani* strain. All the compounds show superior anti-leishmanial activity (IC_{50} = 0.0265 - 1.9146 µg/ml) compared to the standard drug miltefosine (IC_{50} = 3.1911 µg/ml). In particular, compound 4a is a potential lead compound (IC_{50} = 0.0265 µg/ml) exhibited strongest anti-leishmanial activity; 120 and 2 folds more activity as compared to standard drug miltefosine and amphotericin B phosphate (IC_{50} = 0.0460 µg/ml) respectively. Moreover, among the quinoline containing hybrids (5a-c), compound 5c (IC_{50} = 0.1862 µg/ml) shows potent activity next to compound 5a with 17 folds better activity compared to miltefosine. *In vivo* acute toxicity test of these most active compounds show no sign of toxicity.

Keywords: *2,3-Disubstituted-quinazoline-4(3H)-one; 2-pyrazolyl-quinazoline-4(3H)-one hybrid; 2-quinolinyl-quinazoline-4(3H)-one hybrid; anti leishmanial activity*

Introduction

Being one of the major tropical infections classified under the six most dangerous tropical diseases [1] leishmaniasis is a protozoal disease caused by parasites belonging to at least 20 species of the genus leishmania [2]. Worldwide, burden of this disease is revolving in the atmosphere of over 88 countries [3,4]. Currently, there are around 350 million people considered as victims of leishmaniasis with 2 million new cases annually [4,5]. For more than half a century, pentavalent antimonials such as Pentostam (1) and Glucantime (2), alternative drugs such as Amphotericin B (3), Paromomycin (4), and Miltefosine (5) have been the cornerstone of anti-leishmanial chemotherapy [6-9].

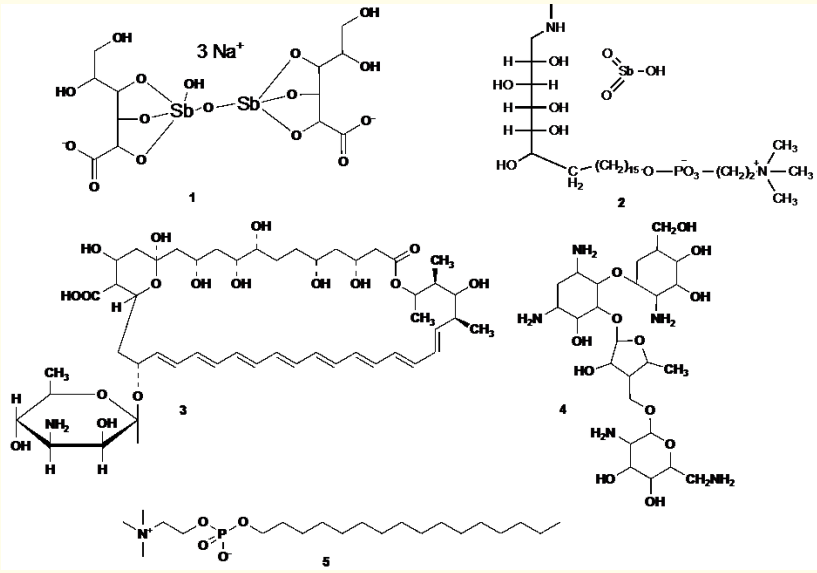


Figure 1:

1. Pentostam
2. Glucantime
3. Amphotericin B
4. Paromomycin
5. Miltefosine

Despite the availability, general use of these drugs has declined due to low efficacy, drug resistance [10] and high toxicity [8,11]. Thus, the lack of generally effective anti-leishmanial drugs ensures the crucial need for developing new, effective, cheap and safe drugs in the field of anti-leishmanial chemotherapy. Recent advances in identifying, designing and developing new chemical derivatives as promising anti-leishmanial agents have become an interesting field. In this pursuit, 2,3-disubstituted-quinazolinones have been continuously presented to have diversified biological activities including anti-leishmanial activities [12,13]. Quinazoline-4(3H)-one is a core scaffold of natural alkaloids with potent anti-leishmanial activity such as vasicinone (6) and tryptanthrin (7) [14]. Substitution of this scaffold at 3-position with aryl groups as in 3-aryl-quinazoline-4(3H)-ones [15], at 2-position as in 3-benzyl-2-phenylquinazolin-4(3H)-one (8) [13] and 3-amino-2-aryl-quinazoline-4(3H)-ones (9) [16] led to series of derivatives with potent anti-leishmanial activity. On the other hand, pyrazole and pyrazole bearing compounds have displayed a broad spectrum of potential pharmacological activities such as analgesic, antipyretic, anti-inflammatory, insecticidal, uricosuric, anticancer, antitumor including anti-leishmanial activity [17,18]. Moreover, one of the privileged moieties for its anti-malarial activity; quinoline has been reported to have anti-malarial, antifungal, antibacterial and anti-leishmanial activities [19,20]. Furthermore, synthesis of new pharmacophore which structurally combines these biologically active moieties has been used as an effective strategy in designing derivatives with enhanced pharmacological activity. For example, Pyrazole bearing 4(3H)-quinazolinones have been found to possess antimicrobial activity [21,22].

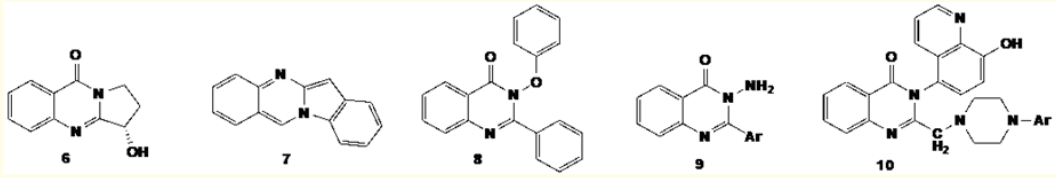


Figure 2:

6. Vasicinone
7. Tryptanthrin
8. 3-benzyl-2-phenylquinazolin-4(3H)-one
9. 3-amino-2-aryl-quinazoline-4(3H)-one
10. Quinazoline-4-one-8-hydroxyquinoline

Whereas, quinoline bearing quinazolinone hybrids as in case of quinazoline-4-one-8-hydroxyquinoline (10) [23] and metal (II) complexes with schiff bases of 2,3-disubstituted-quinazoline-4(3H)-ones [24] have been found to have potent antifungal and antibacterial activities. However, anti-leishmanial activity of these hybrid scaffolds has not been investigated yet. This prompted us to synthesize some new pyrazole and quinoline bearing 2,3-disubstituted-quinazoline-4(3H)-ones and evaluate the effect of their hybrid system for enhanced anti-leishmanial activity.

Materials and Methods

Materials and Instruments

All chemicals and reagents used were obtained from department of Chemistry, Faculty of Science, Addis Ababa University (AAU) and some donated from DDC (Drug Discovery Center), Department of pharmaceutical chemistry, Faculty of Pharmacy, Alexandria University.

For all the synthesized compounds, melting points (°C) were determined in open capillaries using Electrothermal (9100) apparatus and values are uncorrected. IR spectra (Nujol, λmax: cm^{-1}) were recorded on a SHIMADZU 8400SP FT-IR spectrophotometer. H NMR spectra reported in δ (ppm) were recorded on Bruker Avance DMX400 FT-NMR spectrometer using tetramethylsilane (TMS) as internal standard and deutero chloroform ($CDCI_3$) as a solvent. Elemental microanalyses were carried out on Perkin Elmer 2400 elemental analyzer. Silica gel TLC plates of 0.25 mm thickness were used for chromatographic analysis and spots were visualized using iodine vapour and UV radiation.

Test animals and strains

Swiss albino mice of both sex weighing 26-23g and age of 6-8 weeks were obtained from School of Pharmacy, AAU and used for acute toxicity test. The mice were acclimatized for a period of 7 days at appropriate environmental condition. The animals were housed in standard cages and maintained on standard pellated diet and water [27,28]. *L. donovani* isolate (CL/039/09) that causes visceral leishmaniasis in Africa especially in Ethiopia was obtained from Leishmania Diagnosis and Research Laboratory (LDRL) culture bank, School of Medicine, AAU.

Culture medium for anti-leishmanial activity

RPMI-1640 (Gibco, Invitrogen Co., UK), 10% heat- inactivated fetal calf serum (HIFCS), penicillin-streptomycin and 1% L-glutamine all from Sigma Chem. Co., St. Louis, USA were supplied to make complete culture mediums.

Reference drugs

Miltefosine/hexadcylephosphocholine (AG Scientific, San Diego, CA, USA) and amphotericin B deoxycholate (Fungizone®, ER Squibb, UK) were included as reference drugs (positive control) in the *in vitro* anti-leishmanial activity testing of the synthesized compounds.

Stock solution and working concentration preparation for anti-leishmanial activity assay

All the compounds to be evaluated for their anti-leishmanial activity were dissolved in Dimethyl sulfoxide (DMSO) to a final concentration of 1mg/ml. Both test and standard solutions were serially diluted to appropriate concentrations using complete media. The test compounds were prepared by three fold serial dilutions from 10 µg/ml to 0.04 µg/ml. Amphotericin B deoxycholate and miltefosine which were used as a positive control for comparison of the anti-leishmanial activities of the test compounds, were also made in three fold serial dilutions [29,30].

***In vitro* anti-leishmanial activity test**

Promastigote forms of L. donovani and standard drugs of amphotericin B deoxycholate and miltefosine were used for the assay. 3 x 106 promastigotes of L. donovani in 100 µl were seeded to each well in a 96 well flat bottom plate. Various dilutions of test compounds (10, 3.33, 1.11, 0.37, 0.12, 0.04 µg/ml) were added to the parasites. The tests were done in duplicates. Some of the wells contained only the parasites and served as a positive control. The media and DMSO alone acted as a negative control. The plates were then kept at room temperature. After 24h, 20 µl of alamar blue (12.5 mg resazurin dissolved in 100 ml of distilled water) [31] was added to each of the wells. Absorbance of the resulting mixture was measured after 48 hr at a wavelength of 540 and 630 nm using Enzyme Linked Immuno Sorbent Assay (ELISA) plate reader [32].

***In vivo* acute toxicity test**

The most active compounds 4a, 5b and 5c were tested for oral acute toxicity in mice. Four groups of mice, each consisting of six male mice (26-32g) were used to test the acute toxicity. All mice were fasted over night and weighed before test. Test compound was prepared in suspension form in aqueous vehicle containing 7% tween 80 and 3% ethanol [33]. Mice in group one, two and three were given 50, 100 and 200 mg/kg/day of test compound and the fourth group was treated with the vehicle (control group) at a maximum dose of 1 ml/100 g of body weight by oral route. After administration of the substance, food was withheld for a further 2h period (38). The mice were observed closely during the first 30 minutes after dosing, periodically during the first 24h with special attention to the first 4 hr and once daily thereafter for a total of 7 days. The mice were observed for toxicity signs such as changes in skin, blinking eyes, tremors, convulsion, lacrimation, muscle weakness, sedation, urination, salivation, diarrhea, lethargy, sleep, coma and also death. Twenty-four hours later, the weight of test mice in each group was recorded.

Ethical Statement

The *in vivo* acute toxicity test which involves live mice was performed in compliance with ethical guidelines and approval from the National Ethics Review Board of Ethiopia. And ethical clearance was obtained from Addis Ababa University, College of Health Sciences, School of Pharmacy, Department of Pharmacology.

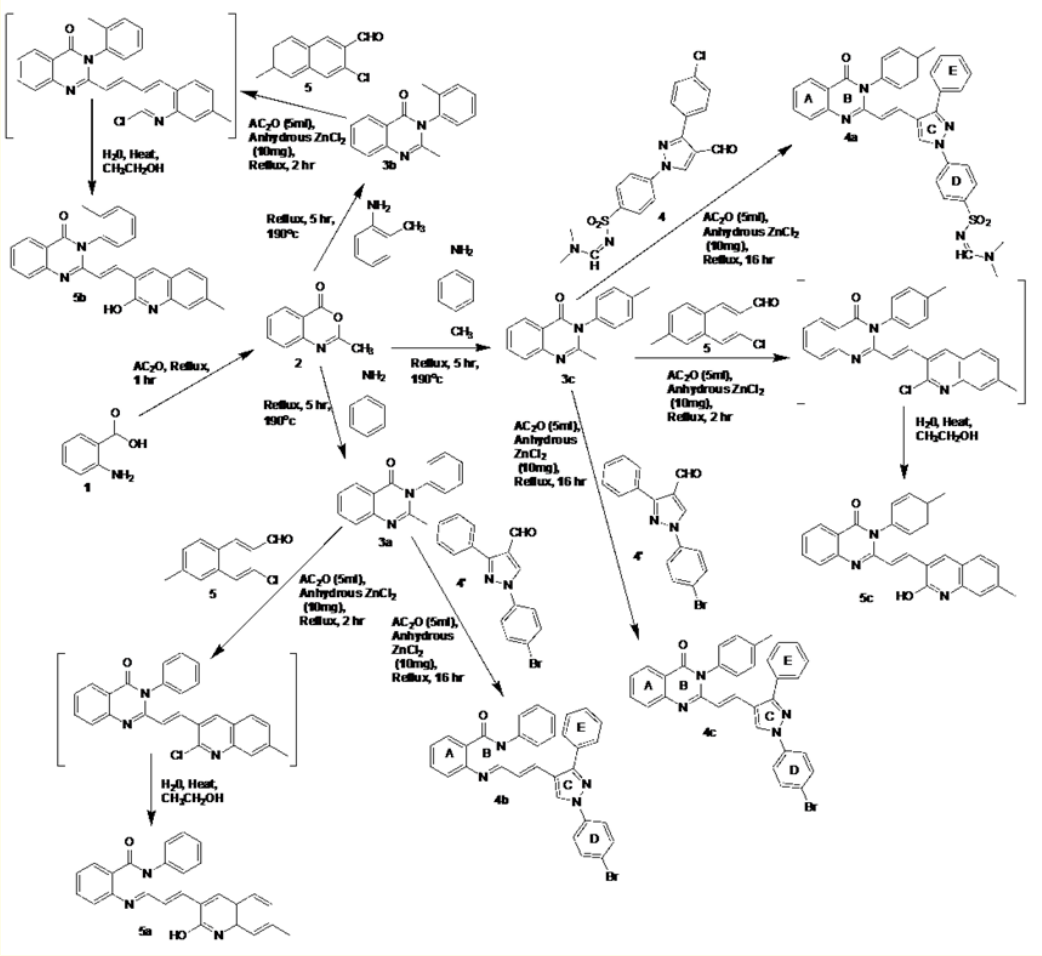
Data analysis

The IC_{50} values for synthesized compounds tested for their *in vitro* anti-leishmanial activity were evaluated from sigmoidal dose- response curves using non linear regression software (GraphPad Prism®; GraphPad Software, Inc., San Diego, CA.

Results and Discussion

Chemistry

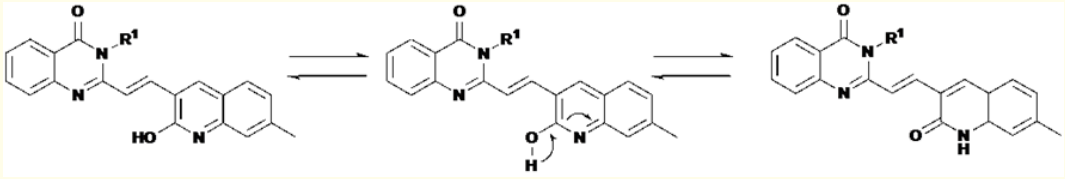
The target compounds (4a-c) and (5a-c) were synthesized following reported methods via condensation reaction of 3-aryl-2-methyl-4(3H)-quinazolinones (3a-c) with 1,3-substituted-1H-pyrazole-4-carboxaldehydes (4 and 4') and 2-chloro-7-methylquinolin-3-carbaldehyde (5) respectively (Scheme 1).



Scheme 1: *Synthesis of 3-aryl-2-pyrazolyl-4(3H)-quinazolinones and 3-aryl-2-quinolinyl quinazolin-4(3H)-ones.*

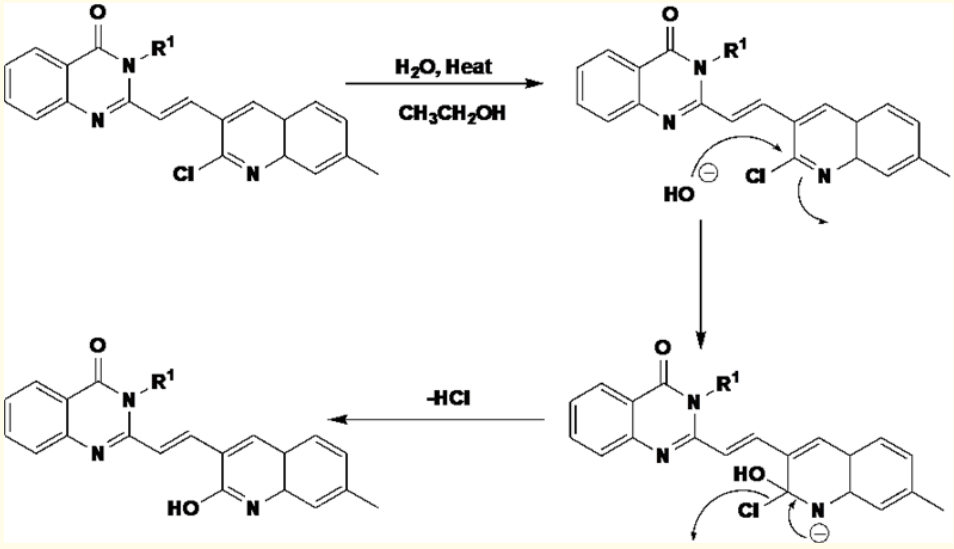
The reaction starts with the thermal transformation of anthranilic acid in to intermediate compound 2 (2-methyl-4-keto-3,4-dihydroquinazoline) due to condensation followed by cyclization reactions in the presence of acetic anhydride. This was accompanied by condensation reaction of the intermediate with aromatic amines to form a series of 2-methyl-3-aryl-quinazolin-4(3H)-ones (3a-c). Further condensation reaction of the second intermediates (3a-c) with 1, 3-substituted-1H-pyrazole-4- carboxaldehydes (4,4') and 2-chloro-7-methyl-quinolin-3-carbaldehyde (5) yielded the target compounds 4a-c and intermediates of 5a-c respectively. Upon heating of the intermediates of 5a-c in the presence of 70% ethanol yielded target compounds 5a-c (Scheme 1).

All the synthesized compounds were synthesized in quantitative yield (68-80%). The compounds were elucidated by spectroscopic measurements (IR, 1H NMR and 13C NMR). The IR spectra of all target compounds showed absorption bands of quinazolinones carbonyl group stretching vibration at 1646-1685 cm^{-1}. Characteristic medium strength bands at 1544 cm^{-1} - 1652 cm^{-1} were assigned to C=N stretching in both the quinazolinone and pyrazole hybrid ring scaffold system of 4a-c compounds. Whereas bands at 1661 cm^{-1} - 1665 cm^{-1} represented carbonyl group formed in unstable tautomeric form of the target compounds 5a-c in which keto-enol tautomerism at the pyridine moiety of quinoline ring system occurs (Scheme 2).



Scheme 2: *Tautomerism of compounds 6a-c.*

The band appeared at low frequency due to the resonance electron donation effect of the adjacent nitrogen atom of the amidic carbonyl group. Absorption band at 3175 cm^{-1} - 3214 cm^{-1} was characteristic to -OH group stretching at the pyridine moiety of 5a-c compounds. Among the significant features of 1H NMR data for compounds 4a-c and 5a-c; the appearance of two doublets peaks at δ 6.17 -8.07 ppm (J = 15.5Hz) was attributed to vinylic protons in (E) configuration. The peaks for the vinylic protons appeared down field due to the presence of electron withdrawing groups surrounding it. In case of compounds 4a-c, the disappearance of singlet peak at up field region for the methyl substituent of quinazolinone moiety at N2- position and a singlet peak at down field region (9-10 ppm) for pyrazole aldehyde proton ensured the formation of the target compounds. In case of compounds 5a-c, the peak at δ 9.5 - 11.2 ppm which appeared as a singlet was correlated to hydroxy group at 2-quinoline position. This proves the nucleophilic aromatic substitution of 2-chloro substituent at the highly electronegative sp2 hybridized quinoline carbon by the highly nucleophilic hydroxy group from the aqueous ethanol which was used as a solvent (Scheme 3). In general, aromatic protons of quinazolinone group appeared as set of multiplet in the region δ 6.24-8.77 ppm, aromatic protons of pyrazole moiety as multiplets in region of δ 7.20-8.10 ppm and phenyl protons of quinoline resonated at δ 6.95 - 7.65 ppm.



Scheme 3: *Nucleophilic substitution reaction of 3-aryl-2-quinolinyl-4(3H)-ones at chlorine atom on the 2- quinoline position by hydroxyl group.*

***In vitro* Anti-leishmanial Activity**

Promastigote forms of *L. donovani* and standard drugs of amphotericin B deoxycholate and miltefosine were used for the assay with alamar blue as a reagent. The IC_{50} values of all tested compounds presented in Table 1 indicate that all the synthesized compounds have by far superior anti-leishmanial activity (0.0265-0.1862 µg/ml) compared to the reference drug miltefosine (IC_{50} = 3.1911 µg/ml). Comparison of IC_{50} values of the compounds revealed that compound 5a (IC_{50} = 0.0265 µg/ml) has the highest activity; having about 120 and 2 folds more activity than the standard drugs miltefosine and amphotericin B deoxycholate (IC_{50} = 0.0460 µg/ml) respectively.

Test Compounds	IC_{50} (µg/ml)
5a	0.0265
5b	1.4217
5c	1.9146
6a	1.2284
6b	0.2764
6c	0.1862
Miltefosine	3.1911
Amphotericin B	0.0460
Deoxycholate	

Table 1: *In vitro antipromastigote activity of the synthesized compounds in IC_{50} (µg/ml).*

These results showed that formation of quinoline and pyrazole bearing quinazoline-4(3H)-one hybrids has definitely positive impact in the enhanced anti-leishmanial activity. This can be justified with previous reports of low anti-leishmanial activity results of 3-alkyl-2-styryl-quinazolone-4(3H)-one derivatives. Particular example is report of Arfan., *et al*. [] 2010, in which 2-Methyl-3-(4-methylphenyl)-quinazolin-4(3H)-ones (11) was synthesized and evaluated to have no activity against Leishmania major. Whereas, introduction of aryl moiety in place of 2-methyl substitute intended to improve the anti-leishmanial activity as in case of 3-aryl-2-styryl-quinazoline-4(3H)-ones. Among which; 3-benzyl-2-phenyl-4(3H)-quinazolinone) (12) showed the highest potency (IC_{50} = 48 µg/mL) against Leishmania major promastigotes and considered as a new anti-leishmanial candidate [13]. In addition, 2-aryl-3-styryl-quinazoline-4(3H)-ones synthesized in our laboratory revealed improved anti-leishmanial activity as in case of (E)-2-(4-hydroxystyryl)-3-p-tolylquinazolin-4(3H)-one (13) with IC_{50} = 1.842 µg/ml which has significantly improved activity compared to 2-Methyl-3-(4-methylphenyl)-quinazolin-4(3H)-ones but still 70 folds less active than compound 5a. These advocate strategies to design quinazolinone-hybrid system with more bulky and lipophilic hetrocyclic scaffolds at 2-postion such as pyrazole and quinoline moieties in order to improve anti-leishmanial activity of 2-aryl-3-alkylaryl-quinazoline-4(3H)-ones.

Furthermore, Para-substitution at phenyl group (ring D) of N^1 of pyrazole ring (ring C) of compounds 4a-c (3-aryl-2-pyrazolyl-quinazolin-4(3H)-one) was critical in determination of their anti-leishmanial activity. The highest activity of compound 4a might be attributed to the presence of para substituent of dimethyl aminomethylene substituted sulphonamide group at ring D which is absent in the other analogues. The most possible reason for this may be the good electron withdrawing effect of Sulphonyl moiety which may involve in delocalization of electrons from aminomethylene group and lead to free radical formation to form covalent bond with some amino acid residues in the parasite such as cysteine proteases. Cysteine proteases and protein kinases are among the most active drug targets against leishmaniasis and their catalytic activity is mostly influenced by lysine, cysteine and aspartic acid residues [25]. From previous SAR reports of substituted quinazoline-4(3H)-ones, carbonyl group and hydroxy group substituents are essential for hydrogen bond interaction with active sites of suggested receptors in leishmania parasites. The aryl groups may act as hydrophobic structural features for van der waal force of interaction with the active site where as dimethyl amino nitrogen may act as a site for hydrogen

bonding. Furthermore, this dimethyl aminomethylene substituted sulphonamide group have been reported before [26] to be essential for anti-leishmanial activity of pyrazole derivatives as in case of (N,N-dimethylaminomethylene-4-[(3-(4-methylphenyl)-4-(phenyl-hydrazonomethylene))-1H-pyrazol-1-yl] benzene sulfonamide (14) (IC_{50} = 0.0175 µg/ml) which is about 180 times more active than miltefosine and 2.7 fold more active than amphotericin B deoxycholate. Replacement of this group with electron withdrawing bromine atom at the 1H-pyrazole-phenyl group as in case of 4b (IC_{50} = 1.4217 µg/ml) and 4c (IC_{50} =1.9146 µg/ml) showed a decrease in anti-leishmanial activity by more than 50 folds. This ensures that dimethyl aminomethylene substituted sulphonamide group is essential for anti-leishmanial activity and needs further docking study.

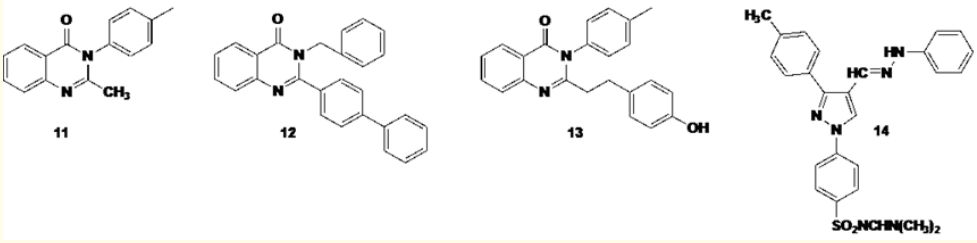


Figure 6:

11. 2-Methyl-3-(4-methylphenyl)-quinazolin-4(3H)-ones

12. 3-benzyl-2-phenyl-4(3H)-quinazolinone

13. (E)-2-(4-hydroxystyryl)-3-p-tolylquinazolin-4(3H)-one

14. (N,N-dimethylaminomethylene-4-[(3-(4-methylphenyl)-4-(phenyl-hydrazonomethylene))-1H-pyrazol-1-yl] benzene sulfonamide

On the other hand; the anti-leishmanial activity test of compounds 5a-c (3-aryl-2-quinolinyl-quinazolin-4(3H)-one derivatives) have shown promising activity. Compound 5c (2-((E)-2-(2-hydroxyquinoline-7-methyl-3-yl)vinyl)-3-o-tolylquinolin-4(3H)-one) (IC50 = 0.1862 µg/ml) has shown potent activity second to compound 4a with 17 folds better activity than miltefosine. Interestingly; methyl substitution at ortho and para position of the aryl substituent exhibited better activity probably due to an increase in liphophilicity of the compounds to penetrate through the membrane of the parasites. In detailed comparison; compound 5c which contained methyl substituent at ortho position showed almost doubled activity than 5b with methyl substituent at para position. This may be pronounced as such groups can enhance interactions with functional groups on the active site of the responsible receptor.

Oral acute toxicity

A preliminary acute toxicity of the most active compounds (4a, 5b and 5c) were evaluated to assess the acute lethal, physical and behavioral changes of these three most active synthesized compounds after administering to mice (weighing 26 to 32g) orally at dose levels of 50 mg/kg, 100 mg/kg and 200 mg/kg. The weight of each mouse was recorded and survival was followed up to 7 days. All the experimental mice did not show any toxicity signs after being treated with test compounds. There was no significant difference in the weight of the mice and no death was recorded during the 7 days after administration of the test compounds. This indicates that the median lethal dose (LD_{50}) of the compounds is much greater than 200 mg/kg. From these results, the test compounds have proved to be non-toxic and well tolerated by the experimental animals up to 200 mg/kg.

Synthesis Chemistry

Synthesis of 2,3-disubstituted-3(4H)-quinazolinone derivatives

Synthesis of 2-methyl-4H-1,3-benzoxazin-4-one (2)

A solution of anthranilic acid 1 (Scheme 1) (15g, 0.09316 mole) in acetic anhydride (38 ml) was heated under reflux for 1h [34,35]. The excess acetic anhydride was evaporated under reduced pressure and the resulting solid mass was obtained which, without purification was suitable for the subsequent reaction. Melting point is 81°C [36].

Synthesis of 3-aryl-2-methyl-4(3H)-quinazolinones (3a-c)

To a separate mixtures of acetanthranil 2 (Scheme 1) (0.1 mole); an equimolar amount of the appropriate aromatic amine (aniline, p-toluidine and o-toluidine) was added. The reaction mixture was heated under reflux at 19°C for 5h, cooled to room temperature and finally recrystalized from ethanol [36-37]. The products obtained were then separated out, filtered, washed with ethanol and air dried.

Synthesis of 3-aryl-2-pyrazolyl-quinazolin-4(3H)-ones (4a-c)

To a mixture of an appropriate 3-aryl-2-methyl-4(3H)-quinazolinone (3a and 3b) (1 mmol) in acetic anhydride (5 ml); an equimolar amount of appropriate pyrazolyl aldehyde 4 and 4' (Scheme 1) obtained by the Vilsmeier–Haack reaction [38,39] was added with 10 mg of anhydrous zinc chloride as catalyst. The reaction mixture was heated under reflux for 16h and set aside at room temperature. The separated yellow product was filtered, dried and recrystalized from ethanol/chloroform in 1:1 mixture.

Synthesis of 3-aryl-2-quinolinyl quinazolin-4(3H)-ones; 5a-c

To a separate mixtures of an appropriate 3-aryl-2-methyl-4(3H)-quinazolinones; 3a-c (1 mmol) in acetic anhydride (5 ml); an equimolar amount of 2-chloro-7-methylquinolin-3-carbaldehyde (5) (scheme 1) synthesized using vilsmeier haack reagent [40,41] was added. Anhydrous zinc chloride (10 mg) was used as a catalyst. The reaction mixture was heated under reflux for 2h. The yellow precipitate formed was filtered, dried and recrystalized from chloroform/ethanol (1:1).

Spectroscopic and elemental analysis data of the synthesized compounds

2-((E)-2-(1-(4-dimethylmethyleneaminosulfonylphenyl)-3-(4-chlorophenyl-1H-pyrazol-4-yl)vinyl)-3-p-tolylquinazolin-4(3H)-one (4a): Yellow solid. Yield: 70%. Mp: 238.0-241.0°C. 1H NMR ($CDCl_3$/$CHCl_3$ δ, ppm): 2.5 (s, 1H, p-tolyl-CH_3), 3.1 (s, 3H, -N-CH_3), 3.2 (s, 3H, -N-CH_3), 6.2 (d, 1H, J = 15.6Hz, vinyl-C_2 H), 7.1 (d, 2H, J = 8.2Hz, p-tolyl-C_3,5 H), 7.35 (d, 2H, J = 8.1Hz, p-tolyl-C2,6 H), 7.4 (d, 2H, J = 8.5Hz, p-chlorophenyl-C_3,5 H), 7.46 (t, 1H, quinazolin-C_7 H), 7.53 (d, 2H, J = 8.5Hz, p-chlorophenyl-C_2,6 H), 7.73-7.84 (m, 4H, 4-dimethylmethylene aminosulphonylphenyl-C_2.6 H and quinazolin-C_6,8 H), 7.94-8.02 (m, 3H, dimethyl methylene aminosulphonylphenyl-C_3,5 H and vinyl-C_1 H), 8.03-8.05 (s, 1H, pyrazolyl-C_5 H) , 8.19 (s, 1H, N=CH) and 8.27 (d, 1H, J = 7.8Hz, quinazolin-C_5 H). 13C NMR ($CDCl_3$/$CHCl_3$) δ ppm: 21.35, 35.61, 41.57, 118.74, 119.4, 120.56, 120.84, 126.53, 127.2, 127.3, 128.16, 128.2, 128.92, 129.26, 129.89, 130.57, 130.74, 134.09, 134.57, 134.80, 139.31, 140.66, 141.51, 147.71, 151.66, 152.22, 159.15, 162.41. Anal. calcd. for $C_{35}H_{29}ClN_6O_3S$: C, 64.76; H, 4.5; Cl, 5.46; N, 12.96; S, 4.94. Found: C, 64.54; H, 4.26; N, 13.21; Cl, 5.14; S, 5.21.

2-((E)-2-(1-(4-bromophenyl)-3-phenyl-1H-pyrazol-4-yl)vinyl)-3-p-tolylquinazolin-4(3H)-one (4b): Yellow solid. Yield: 68%. Mp: 277.0-279.0°C. 1H NMR ($CDCl_3$/$CHCl_3$) ppm: 2.5 (s, 3H, CH_3), 6.22 (d, 1H, J = 15.6 Hz, vinyl-C_2 H), 7.13 (d, 2H, J = 8.2 Hz, p-tolyl-C_3,5 H), 7.35 (t, 3H, phenyl-C_3,4,5 H), 7.44-7.52 (m, 5H, pyrazolylphenyl-C_2,6 H; Phenyl-C_2,6 H and quinazolin-C_7 H), 7.57 (d, 2H, J = 8.423Hz, bromophenyl-C_2,6 H), 7.68-7.82 (m, 4H, 4-bromophenyl)-C_3,5 H; quinazolin-C_6 H and vinyl-C_1 H), 8.0 (m, 2H, quinazolin-C_8 H and Pyrazolyl-C_5 H), 8.3 (d, 1H, J = 7.808Hz, quinazolin-C_5 H). Anal. Calcd for $C_{32}H_{23}BrN_4O$: C, 68.70; H, 4.14; Br, 14.28; N, 10.01. Found: C, 68.99; H, 3.87; Br, 14.56; N, 9.84.

2-((E)-2-(1-(4-bromophenyl)-3-phenyl)-1H-pyrazol-4-yl)vinyl)-3-phenylquinazolin-4(3H)-one(4c): Yellow solid: Yield:69%. Mp: 217-219°C. 1H NMR ($CDCl_3$/$CHCl_3$) ppm: 6.17 (d, 1H, J = 15.5 Hz, vinyl-C_2 H), 7.25-7.28 (m, 2H, pyrazolylphenyl-C_4 H and quinazolinylphenyl C_4 H), 7.35 (t, 1H, quinazolin-C_7 H), 7.45-7.5 (m, 6H, phenyl- C_3,5 H, pyrazolylphenyl-C_3,5 H and bromophenyl-C_2,6 H), 7.54-7.59 (m, 4H, pyrazolylphenyl-C_2,6 H and bromophenyl-C_3,5 H), 7.7 (d, 2H, J = 8.04 Hz, phenyl-C_2,6 H), 7.8 (m, 2H, quinazolin-C_6,8 H), 7.96 (s, 1H, pyrazolyl-C_5 H), 8.02 (d, 1H, J = 15.5 Hz, vinyl-C_2 H), 8.3 (d, 1H, J = 7.3 Hz, quinazolin-C_5 H). Anal. Calcd for $C_{31}H_{21}BrN_4$: C, 68.26; H, 3.88; Br, 14.65; N, 10.27. Found: C, 67.92; H, 4.04; Br, 14.32; N, 10.54.

2-[(E)-2-(2-hydroxyquinoline-7-methyl-3-yl)vinyl]-3-p-tolylquinazolin-4(3H)-one(5a): Yellow solid. Yield: 78%. Mp: 311-313°C. ^{1}H NMR ($CDCl_3$/$CHCl_3$) δ ppm: 2.4 (s, 3H, p-tolyl-4-CH_3), 2.55 (s, 3H, quinolin-7-CH_3), 7.0-7.08 (m, 2H, p-tolyl-C_3,5 H), 7.24 (d, 2H, p-tolyl-C_2,6 H), 7.35-7.52 (m, 4H, vinyl-C_2 H, quinolin-C_5,6 H and quinazolin-C_7 H), 7.81-7.86 (m, 3H, quinazolin-C_6,8 H and quinolin-C_8 H), 8.07 (d, 1H, J = 15.2 Hz, Vinyl-C_1H), 8.34 (d, 1H, J = 7.8 Hz, quinazolin-C_5 H) and 10.9 (s, 1H, quinolin-C_2 OH enol form). Anal. Calcd. For $C_{27}H_{20}ClN_3O$, C, 77.31; H, 5.05; N, 10.02: Found C, 77.26; H, 4.82; N, 10.38.

2-[(E)-2-(2-hydroxy-7-methylquinolin-3-yl)vinyl)-3-phenylquinazolin-4(3H)-one(5b): Yellow solid: Yield: 80%. Mp: 321-324°C. 1H NMR ($CDCl_3$/$CHCl_3$) δ ppm: 2.5 (s, 3H, CH_3), 7.06 (d, 1H, J = 15.2 Hz, vinyl-C_2 H), 6.9 (s, 1H, quinolin-C_4 H), 7.35-7.64 (m, 8H, phenyl-C_3,4,5 H, quinazolin-C_6,7 and quinolin-C_5,6,8 H), 7.8-7.85 (m, 3H, quinazolin-C_8 H and phenyl-C_2,6 H), 8.0 (d, 1H, J = 15.2 Hz, vinyl-C1 H), 8.35 (d, 1H, J = 7.9 Hz, quinazolin-C_5 H) and 9.5 (s, 1H, quinolin-C_2 OH, D2O exchangeable). Anal. Calcd. For $C_{26}H_{18}ClN_3O$, C, 77.02; H, 4.72; N, 10.36: Found C, 76.85; H, 4.64; N, 10.17.

2-[(E)-2-(2-hydroxy-7-methylquinolin-3-yl)vinyl]-3-o-tolylquinazolin-4(3H)-one(5c); Yellow solid: Yield: 76%. Mp: 276-278°C. 1H NMR ($CDCl_3$/$CHCl_3$) δ ppm: 2.1 (s, 3H, o-tolyl-CH_3), 2.4 (s, 3H, quinolin-7- CH_3), 6.85 (s, 1H, quinolin-C_8 H), 6.95-7.10 (m, 3H, quinolin-C_6 H and o-tolyl-C_3,5 H), 7.15-7.20 (m, 2H, o-tolyl-C_4,6 H), 7.25-7.38 (m, 2H, quinazolin-C_7 H and vinyl-C_1 H), 7.45-7.60 (m, 2H, quinazolin-C_8 H and quinolin-C_5 H), 7.78-7.85 (m, 2H, quinazolin-C_6 H and quinolin-C_4 H), 7.88-7.95 (d, 1H, J = 7.783Hz, vinyl-C_2 H), 8.31 (d, 1H, J = 15.078Hz, quinazolin-C_5 H), and 11.2 (s, 1H, -N=C-OH). Anal. Calcd. For $C_{27}H_{20}ClN_3O$, C, 77.31; H, 5.05; N, 10.02: Found C, 77.81; H, 5.44; N, 10.42.

Conclusion

In this study, six new pyrazolyl and quinolinyl hybrids of 2,3-disubstituted-quinazoline-4(3H)-ones were synthesized and evaluated for their anti-leishmanial activity against Leishmania donovani *in vitro*. Among the synthesized compounds, compound 4a exhibited strongest anti-leishmanial activity; 120 folds more activity than miltefosine. In addition, from compounds bearing quinoline moiety, compound 5c has shown potent activity second to compound 4a with 17 folds better activity than miltefosine. These properties highlighted that quinazolinone hybrid system with biologically active pyrazole and quinoline moieties have shown promising anti-leishmanial activity and enhance further study depending on this strategy. Detail SAR and molecular docking study of these compounds can lead to important results in identification of molecular targets of these compounds and can be applied to design quinazolinone hybrid system with potent anti-leishmanial activity. Acute toxicity test of these two more active compounds have also shown no sign of toxicity.

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